Docket No. 60390-IA/JPW/GJG/JBC

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that

Arlindo L. Castelhano, Bryan McKibben and David J. Witter

have invented certain new and useful improvements in

COMPOUNDS SPECIFIC TO ADENOSINE A_1 RECEPTORS AND USES THEREOF

of which the following is a full, clear and exact description.

COMPOUNDS SPECIFIC TO ADENOSINE A_1 RECEPTORS AND USES THEREOF

This application is a continuation of U.S. Application No. 10/000,280, filed November 30, 2001, which claims the benefit of U.S. Provisional Application No. 60/250,895, filed December 1, 2000, the entire contents of which are hereby incorporated by reference.

Background of the Invention

Adenosine is an ubiquitous modulator of numerous physiological activities, particularly within the cardiovascular and nervous systems. The effects of adenosine appear to be mediated by specific cell surface receptor proteins. Adenosine modulates diverse physiological functions including induction of sedation, vasodilation, suppression of cardiac rate and contractility, inhibition of platelet aggregability, stimulation of gluconeogenesis and inhibition of lipolysis. In addition to its effects on adenylate cyclase, adenosine has been shown to open potassium channels, reduce flux through calcium channels, and inhibit or stimulate phosphoinositide turnover through receptor-mediated mechanisms (See for example, C.E. Muller and B. Stein "Adenosine Receptor Antagonists: Structures and Potential Therapeutic Applications," *Current Pharmaceutical Design*, **2**:501 (1996) and C.E. Muller "A_1-Adenosine Receptor Antagonists," *Exp. Opin. Ther. Patents* **7**(5):419 (1997)).

Adenosine receptors belong to the superfamily of purine receptors which are currently subdivided into P_1 (adenosine) and P_2 (ATP, ADP, and other nucleotides) receptors. Four receptor subtypes for the nucleoside adenosine have been cloned so far from various species including humans. Two receptor subtypes (A_1 and A_{2a}) exhibit affinity for adenosine

in the nanomolar range while two other known subtypes A_{2b} and A_3 are low-affinity receptors, with affinity for adenosine in the low-micromolar range. A_1 and A_3 adenosine receptor activation can lead to an inhibition of adenylate cyclase activity, while A_{2a} and A_{2b} activation causes a stimulation of adenylate cyclase.

A few A_1 antagonists have been developed for the treatment of cognitive disease, renal failure, and cardiac arrhythmias. It has been suggested that A_{2a} antagonists may be beneficial for patients suffering from Morbus Parkinson (Parkinson's disease). Particularly in view of the potential for local delivery, adenosine receptor antagonists may be valuable for treatment of allergic inflammation and asthma. Available information (for example, Nyce & Metzger "DNA antisense Therapy for Asthma in an Animal Model" *Nature* (1997) 385: 721-5)indicates that in this pathophysiologic context, A_1 antagonists may block contraction of smooth muscle underlying respiratory epithelia, while A_{2b} or A_3 receptor antagonists may block mast cell degranulation, mitigating the release of histamine and other inflammatory mediators. A_{2b} receptors have been discovered throughout the gastrointestinal tract, especially in the colon and the intestinal epithelia. It has been suggested that A_{2b} receptors mediate cAMP response (Strohmeier *et al.*, *J. Bio. Chem.* (1995) 270:2387-94).

Adenosine receptors have also been shown to exist on the retinas of various mammalian species including bovine, porcine, monkey, rat, guinea pig, mouse, rabbit and human (See, Blazynski *et al.*, "Discrete Distributions of Adenosine Receptors in Mammalian Retina," *Journal of Neurochemistry*, volume 54, pages 648-655 (1990); Woods *et al.*, "Characterization of Adenosine A_1-Receptor Binding Sites in Bovine Retinal Membranes," *Experimental Eye Research*, volume 53, pages 325-331 (1991); and Braas *et al.*, "Endogenous

adenosine and adenosine receptors localized to ganglion cells of the retina," *Proceedings of the National Academy of Science*, volume 84, pages 3906-3910 (1987)). Recently, Williams reported the observation of adenosine transport sites in a cultured human retinal cell line (Williams *et al.*, "Nucleoside Transport Sites in a Cultured Human Retinal Cell Line Established By SV-40 T Antigen Gene," *Current Eye Research*, volume 13, pages 109-118 (1994)).

Compounds which regulate the uptake of adenosine have previously been suggested as potential therapeutic agents for the treatment of retinal and optic nerve head damage. In U.S. Patent No. 5,780,450 to Shade, Shade discusses the use of adenosine uptake inhibitors for treating eye disorders. Shade does not disclose the use of specific A_3 receptor inhibitors. The entire contents of U.S. Patent No. 5,780,450 are hereby incorporated herein by reference.

Additional adenosine receptor antagonists are needed as pharmacological tools and are of considerable interest as drugs for the above-referenced disease states and/or conditions.

Summary of the Invention

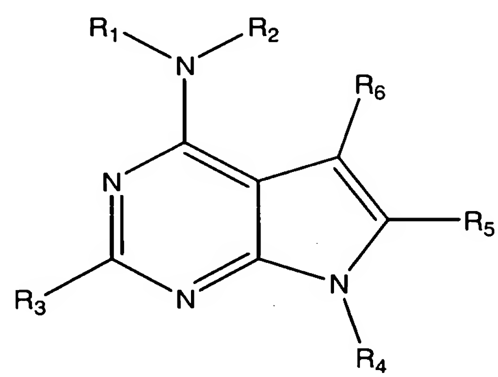
The present invention is based on compounds which selectively bind to adenosine A_1 receptor, thereby treating a disease associated with A_1 adenosine receptor in a subject by administering to the subject a therapeutically effective amount of such compounds. The diseases to be treated are associated with cognitive disease, renal failure, cardiac arrhythmias, respiratory epithelia, transmitter release, sedation, vasoconstriction, bradycardia, negative cardiac inotropy and dromotropy, branchoconstriction, neutropil chemotaxis, reflux condition, or ulcerative condition.

The present invention is based, at least in part, on the discovery that certain N-6 substituted 7-deazapurines, described *infra*, can be used to treat a N-6 substituted 7-deazapurine responsive state. Examples of such states include those in which the activity of the adenosine receptors is increased, *e.g.*, bronchitis, gastrointestinal disorders, or asthma. These states can be characterized in that adenosine receptor activation can lead to the inhibition or stimulation of adenylate cyclase activity. Compositions and methods of the invention include enantiomerically or diastereomerically pure N-6 substituted 7-deazapurines. Preferred N-6 substituted 7-deazapurines include those which have an acetamide, carboxamide, substituted cyclohexyl, *e.g.*, cyclohexanol, or a urea moiety attached to the N-6 nitrogen through an alkylene chain.

The present invention pertains to methods for modulating an adenosine receptor(s) in a mammal by administering to the mammal a therapeutically effective amount of a N-6 substituted 7-deazapurine, such that modulation of the adenosine receptor's activity occurs. Suitable adenosine receptors include the families of A_1, A_2, or A_3 receptors. In a preferred embodiment, the N-6 substituted 7-deazapurine is an

adenosine receptor antagonist.

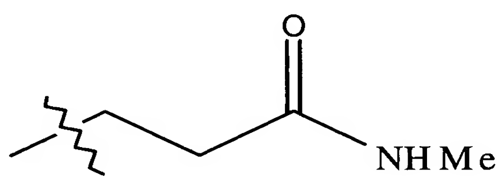
The invention further pertains to methods for treating N-6 substituted 7-deazapurine disorders, *e.g.*, asthma, bronchitis, allergic rhinitis, chronic obstructive pulmonary disease, renal disorders, gastrointestinal disorders, and eye disorders, in a mammal by administering to the mammal a therapeutically effective amount of a N-6 substituted 7-deazapurine, such that treatment of the disorder in the mammal occurs. Suitable N-6 substituted 7 deazapurines include those illustrated by the general formula I:



(I)

and pharmaceutically acceptable salts thereof. R_1 and R_2 are each independently a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or together form a substituted or unsubstituted heterocyclic ring. R_3 is a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety. R_4 is a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety. R_5 and R_6 are each independently a halogen atom, *e.g.*, chlorine, fluorine, or bromine, a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or R_5 is carboxyl, esters of carboxyl, or carboxamides, or R_4 and R_5 or R_5 and R_6 together form a substituted or unsubstituted heterocyclic or carbocyclic ring.

In certain embodiments, R_1 and R_2 can each independently be a substituted or unsubstituted cycloalkyl or heteroarylalkyl moieties. In other embodiments, R_3 is a hydrogen atom or a substituted or unsubstituted heteroaryl moiety. In still other embodiments, R_4, R_5 and R_6 can each independently be heteroaryl moieties. In a preferred embodiment, R_1 is a hydrogen atom, R_2 is a cyclohexanol, *e.g.*, *trans*-cyclohexanol, R_3 is phenyl, R_4 is a hydrogen atom, R_5 is a methyl group and R_6 is a methyl group. In still another embodiment, R_1 is a hydrogen atom, R_2 is



,

R_3 is phenyl, R_4 is a hydrogen atom and R_5 and R_6 are methyl groups.

The invention further pertains to pharmaceutical compositions for treating a N-6 substituted 7-deazapurine responsive state in a mammal, *e.g.*, asthma, bronchitis, allergic rhinitis, chronic obstructive pulmonary disease, renal disorders, gastrointestinal disorders, and eye disorders. The pharmaceutical composition includes a therapeutically effective amount of a N-6 substituted 7-deazapurine and a pharmaceutically acceptable carrier.

The present invention also pertains to packaged pharmaceutical compositions for treating a N-6 substituted 7-deazapurine responsive state in a mammal. The packaged pharmaceutical composition includes a container holding a therapeutically effective amount of at least one N-6 substituted 7-deazapurine and instructions for using the N-6 substituted 7-deazapurine for treating a N-6 substituted 7-deazapurine responsive state

in a mammal.

The invention further pertains to compounds of formula I wherein

R_1 is hydrogen;

R_2 is substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkyl, or R_1 and R_2 together form a substituted or unsubstituted heterocyclic ring;

R_3 is unsubstituted or substituted aryl;

R_4 is hydrogen; and

R_5 and R_6 are each independently hydrogen or alkyl, and pharmaceutically acceptable salts thereof. The deazapurines of this embodiment may advantageously be selective A_3 receptor antagonists. These compounds may be useful for numerous therapeutic uses such as, for example, the treatment of asthma, kidney failure associated with heart failure, and glaucoma. In a particularly preferred embodiment, the deazapurine is a water soluble prodrug that is capable of being metabolized *in vivo* to an active drug by, for example, esterase catalyzed hydrolysis.

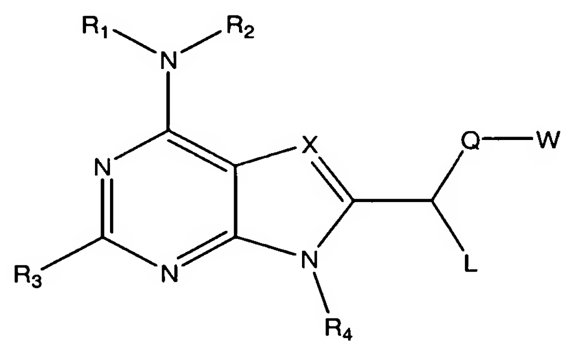
In yet another embodiment, the invention features a method for inhibiting the activity of an adenosine receptor (*e.g.*, A_3) in a cell, by contacting the cell with N-6 substituted 7-deazapurine (*e.g.*, preferably, an adenosine receptor antagonist).

In another aspect, the invention features a method for treating damage to the eye of an animal(*e.g.*, a human) by administering to the animal an effective amount of an N-6 substituted 7-deazapurine of formula I. Preferably, the N-6 substituted 7-deazapurine is an antagonist of A_3 adenosine receptors in cells of the animal. The damage is to the retina or the optic nerve head and may be acute or chronic. The damage may be the result of, for example, glaucoma, edema,

ischemia, hypoxia or trauma.

The invention also features a pharmaceutical composition comprising a N-6 substituted compound of formula I. Preferably, the pharmaceutical preparation is an ophthalmic formulation (*e.g.*, an periocular, retrobulbar or intraocular injection formulation, a systemic formulation, or a surgical irrigating solution).

In yet another embodiment, the invention features a compound having the formula II:



(II)

wherein X is N or CR_6; R_1 and R_2 are each independently hydrogen, or substituted or unsubstituted alkoxy, aminoalkyl, alkyl, aryl, or alkylaryl, or together form a substituted or unsubstituted heterocyclic ring, provided that both R_1 and R_2 are both not hydrogen; R_3 is substituted or unsubstituted alkyl, arylalkyl, or aryl; R_4 is hydrogen or substituted or unsubstituted C_1-C_6 alkyl; L is hydrogen, substituted or unsubstituted alkyl, or R_4 and L together form a substituted or unsubstituted heterocyclic or carbocyclic ring; R_6 is hydrogen, substituted or unsubstituted alkyl, or halogen; Q is CH_2, O, S, or $NR_{7'}$, wherein $R_{7'}$ is hydrogen or substituted or unsubstituted C_1-C_6 alkyl; and W is unsubstituted or

substituted alkyl, cycloalkyl, aryl, arylalkyl, biaryl, heteroaryl, substituted carbonyl, substituted thiocarbonyl, or substituted sulfonyl; provided that if R_3 is pyrrolidino, then R_4 is not methyl. The invention also pertains to pharmaceutically acceptable salts and prodrugs of the compounds of the invention.

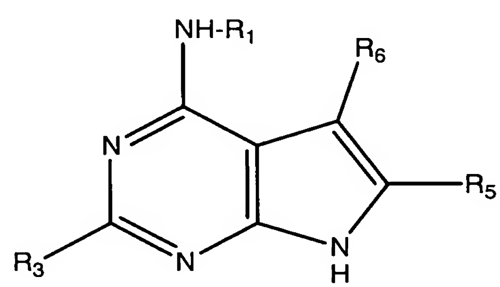
In an advantageous embodiment, X is CR_6 and Q is CH_2, O, S, or NH in formula II, wherein R_6 is as defined above.

In another embodiment of formula II, X is N.

The invention further pertains to a method for inhibiting the activity of an adenosine receptor (*e.g.*, an A_{2b} adenosine receptor) in a cell by contacting the cell with a compound of the invention. Preferably, the compound is an antagonist of the receptor.

The invention also pertains to a method for treating a gastrointestinal disorder (*e.g.*, diarrhea) or a respiratory disorder (*e.g.*, allergic rhinitis, chronic obstructive pulmonary disease) in an animal by administering to an animal an effective amount of a compound of formula II (*e.g.*, an antagonist of A_{2b}). Preferably, the animal is a human.

This invention also features a compound having the structure:

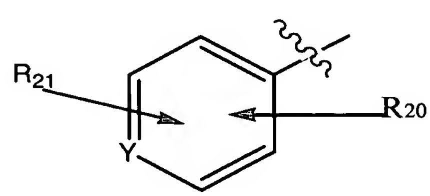


IV

wherein R_1 is *trans*-4-hydroxy cyclohexyl, 2-methylamino carbonylamino cyclohexyl, acetamido ethyl, or methylamino carbonylamino ethyl;

wherein R_3 is a substituted or unsubstituted four to six membered ring.

In one embodiment of the compound, R_3 is phenyl, pyrrole, thiophene, furan, thiazole, imidazole, pyrazole, 1,2,4-triazole, pyridine, 2(1H)-pyridone, 4(1H)-pyridone, pyrazine, pyrimidine, pyridazine, isothiazole, isoxazole, oxazole, tetrazole, naphthalene, tetralin, naphthyridine, benzofuran, benzothiophene, indole, 2,3-dihydroindole, 1H-indole, indoline, benzopyrazole, 1,3-benzodioxole, benzoxazole, purine, coumarin, chromone, quinoline, tetrahydroquinoline, isoquinoline, benzimidazole, quinazoline, pyrido[2,3-b]pyrazine, pyrido[3,4-b]pyrazine, pyrido[3,2-c]pyridazine, pyrido[3,4-b]-pyridine, 1H-pyrazole[3,4-d]pyrimidine, pteridine, 2(1H)-quinolone, 1(2H)-isoquinolone, 1,4-benzisoxazine, benzothiazole, quinoxaline, quinoline-N-oxide, isoquinoline-N-oxide, quinoxaline-N-oxide, quinazoline-N-oxide, benzoxazine, phthalazine, cinnoline, or having a structure:



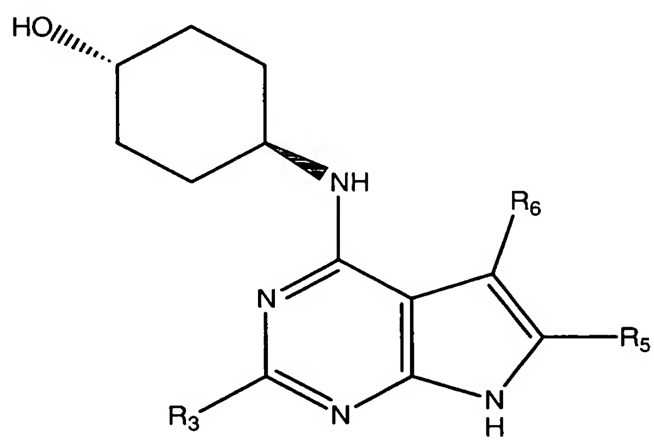
wherein Y is carbon or nitrogen;

wherein R_{20} and R_{21} are independently H, substituted or unsubstituted alkyl, substituted or unsubstituted aryl, halogen, methoxy, methyl amino, or methyl thio;

wherein R_5 is H, alkyl, substituted alkyl, aryl, arylalkyl, amino, substituted aryl, wherein said substituted alkyl is $-C(R_7)(R_8)XR_9$, wherein X is O, S, or NR_{10}, wherein R_7 and R_8 are each independently H or alkyl, wherein R_9 and R_{10} are each independently alkyl or cycloalkyl, or R_9, R_{10} and the nitrogen together form a substituted or unsubstituted ring of between 4 and 7 members;

wherein R_6 is H, alkyl, substituted alkyl, cycloalkyl; or a pharmaceutically acceptable salt, or a prodrug derivative, or a biologically active metabolite; with the proviso that when R_1 is acetylamino ethyl, R_3 is not 4-pyridyl.

This invention also pertains to a compound having the structure:



V

wherein R_3 is aryl, substituted aryl, or heteroaryl;

wherein R_5 is H, alkyl, substituted alkyl, aryl, arylalkyl, amino, substituted aryl, wherein said substituted alkyl is $-C(R_7)(R_8)NR_9R_{10}$, wherein R_7 and R_8 are each H or alkyl, wherein R_9 and R_{10} are each alkyl or cycloalkyl, or R_9, R_{10} and the nitrogen together form a ring system of between 4 and 7 members; and

wherein R_6 is H, alkyl, substituted alkyl, or cycloalkyl. This invention also features a method for inhibiting the

activity of an A_1 adenosine receptor in a cell, which comprises contacting said cell with the above-mentioned compounds.

Detailed Description

The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

The present invention pertains to methods for treating a N-6 substituted 7-deazapurine responsive state in a mammal. The methods include administration of a therapeutically effective amount of a N-6 substituted 7-deazapurine, described *infra*, to the mammal, such that treatment of the N-6 substituted 7-deazapurine responsive state in the mammal occurs.

The language "N-6 substituted 7-deazapurine responsive state" is intended to include a disease state or condition characterized by its responsiveness to treatment with a N-6 substituted 7-deazapurine of the invention as described *infra*, *e.g.*, the treatment includes a significant diminishment of at least one symptom or effect of the state achieved with a N-6 substituted 7-deazapurine of the invention. Typically such states are associated with an increase of adenosine within a host such that the host often experiences physiological symptoms which include, but are not limited to, release of toxins, inflammation, coma, water retention, weight gain or weight loss, pancreatitis, emphysema, rheumatoid arthritis, osteoarthritis, multiple organ failure, infant and adult respiratory distress syndrome, allergic rhinitis, chronic obstructive pulmonary disease, eye disorders, gastrointestinal disorders, skin tumor promotion, immunodeficiency and asthma. (See for example, C.E. Muller and B. Stein "Adenosine Receptor Antagonists: Structures and Potential Therapeutic Applications," *Current Pharmaceutical Design*, **2**:501 (1996) and

C.E. Muller "A_1-Adenosine Receptor Antagonists," *Exp. Opin. Ther. Patents* **7**(5):419 (1997) and I. Feoktistove, R. Polosa, S. T. Holgate and I. Biaggioni "Adenosine A_{2B} receptors: a novel therapeutic target in asthma?" TiPS **19**; 148 (1998)). The effects often associated with such symptoms include, but are not limited to, fever, shortness of breath, nausea, diarrhea, weakness, headache, and even death. In one embodiment, a N-6 substituted 7-deazapurine responsive state includes those disease states which are mediated by stimulation of adenosine receptors, *e.g.*, A_1, A_{2a}, A_{2b}, A_3, etc., such that calcium concentrations in cells and/or activation of PLC (phospholipase C) is modulated. In a preferred embodiment, a N-6 substituted 7-deazapurine responsive state is associated with adenosine receptor(s), *e.g.*, the N-6 substituted 7-deazapurine acts as an antagonist. Examples of suitable responsive states which can be treated by the compounds of the invention, *e.g.*, adenosine receptor subtypes which mediate biological effects, include central nervous system (CNS) effects, cardiovascular effects, renal effects, respiratory effects, immunological effects, gastro-intestinal effects and metabolic effects. The relative amount of adenosine in a subject can be associated with the effects listed below; that is, increased levels of adenosine can trigger an effect, *e.g.*, an undesired physiological response, *e.g.*, an asthmatic attack.

CNS effects include decreased transmitter release (A_1), sedation (A_1), decreased locomotor activity (A_{2a}), anticonvulsant activity, chemoreceptor stimulation (A_2) and hyperalgesia. Therapeutic applications of the inventive compounds include treatment of dementia, Alzheimer's disease and memory enhancement.

Cardiovascular effects include vasodilation (A_{2a}), (A_{2b}) and

(A_3), vasoconstriction (A_1), bradycardia (A_1), platelet inhibition (A_{2a}), negative cardiac inotropy and dromotropy (A_1), arrhythmia, tachycardia and angiogenesis. Therapeutic applications of the inventive compounds include, for example, prevention of ischaemia-induced impairment of the heart and cardiotonics, myocardial tissue protection and restoration of cardiac function.

Renal effects include decreased GFR (A_1), mesangial cell contraction (A_1), antidiuresis (A_1) and inhibition of renin release (A_1). Suitable therapeutic applications of the inventive compounds include use of the inventive compounds as diuretic, natriuretic, potassium-sparing, kidney-protective/prevention of acute renal failure, antihypertensive, anti-oedematous and anti-nephritic agents.

Respiratory effects include bronchodilation (A_2), bronchoconstriction (A_1), chronic obstructive pulmonary disease, allergic rhinitis, mucus secretion and respiratory depression (A_2). Suitable therapeutic applications for the compounds of the invention include anti-asthmatic applications, treatment of lung disease after transplantation and respiratory disorders.

Immunological effects include immunosuppression (A_2), neutrophil chemotaxis (A_1), neutrophil superoxide generation (A_{2a}) and mast cell degranulation (A_{2b} and A_3) Therapeutic applications of antagonists include allergic and non allergic inflammation, *e.g.*, release of histamine and other inflammatory mediators.

Gastrointestinal effects include inhibition of acid secretion (A_1) therapeutic application may include reflux and ulcerative conditions. Gastrointestinal effects also include colonic, intestinal and diarrheal disease, *e.g.*, diarrheal disease

associated with intestinal inflammation (A_{2b}).

Eye disorders include retinal and optic nerve head injury and trauma related disorders (A_3). In a preferred embodiment, the eye disorder is glaucoma.

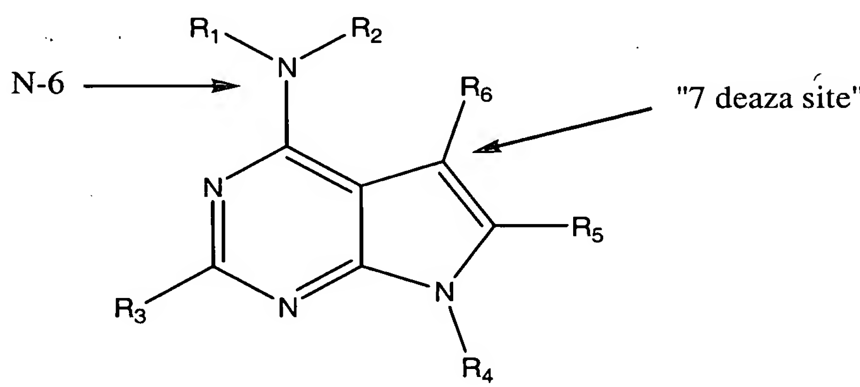
Other therapeutic applications of the compounds of the invention include treatment of obesity (lipolytic properties), hypertension, treatment of depression, sedative, anxiolytic, as antileptics and as laxatives, *e.g.*, effecting motility without causing diarrhea.

The term "disease state" is intended to include those conditions caused by or associated with unwanted levels of adenosine, adenylyl cyclase activity, increased physiological activity associated with aberrant stimulation of adenosine receptors and/or an increase in cAMP. In one embodiment, the disease state is, for example, asthma, chronic obstructive pulmonary disease, allergic rhinitis, bronchitis, renal disorders, gastrointestinal disorders, or eye disorders. Additional examples include chronic bronchitis and cystic fibrosis. Suitable examples of inflammatory diseases include non-lymphocytic leukemia, myocardial ischaemia, angina, infarction, cerebrovascular ischaemia, intermittent claudication, critical limb ischemia, venous hypertension, varicose veins, venous ulceration and arteriosclerosis. Impaired reperfusion states include, for example, any post-surgical trauma, such as reconstructive surgery, thrombolysis or angioplasty.

The language "treatment of a N-6 substituted 7-deazapurine responsive state" or "treating a N-6 substituted 7-deazapurine responsive state" is intended to include changes in a disease state or condition, as described above, such that physiological symptoms in a mammal can be significantly

diminished or minimized. The language also includes control, prevention or inhibition of physiological symptoms or effects associated with an aberrant amount of adenosine. In one preferred embodiment, the control of the disease state or condition is such that the disease state or condition is eradicated. In another preferred embodiment, the control is selective such that aberrant levels of adenosine receptor activity are controlled while other physiologic systems and parameters are unaffected.

The term "N-6 substituted 7-deazapurine" is art recognized and is intended to include those compounds having the formula **I:**



(**I**)

"N-substituted 7-deazapurine" includes pharmaceutically acceptable salts thereof, and, in one embodiment, also includes certain N-6 substituted purines described herein.

In certain embodiments, the N-6 substituted 7-deazapurine is not N-6 benzyl or N-6 phenylethyl substituted. In other embodiments, R_4 is not benzyl or phenylethyl substituted. In preferred embodiments, R_1 and R_2 are both not hydrogen atoms. In still other preferred embodiments, R_3 is not a hydrogen atom.

The language "therapeutically effective amount" of an N-6

substituted 7-deazapurine, described *infra*, is that amount of a therapeutic compound necessary or sufficient to perform its intended function within a mammal, *e.g.*, treat a N-6 substituted 7-deazapurine responsive state, or a disease state in a mammal. An effective amount of the therapeutic compound can vary according to factors such as the amount of the causative agent already present in the mammal, the age, sex, and weight of the mammal, and the ability of the therapeutic compounds of the present invention to affect a N-6 substituted 7-deazapurine responsive state in the mammal. One of ordinary skill in the art would be able to study the aforementioned factors and make a determination regarding the effective amount of the therapeutic compound without undue experimentation. An *in vitro* or *in vivo* assay also can be used to determine an "effective amount" of the therapeutic compounds described *infra*. The ordinarily skilled artisan would select an appropriate amount of the therapeutic compound for use in the aforementioned assay or as a therapeutic treatment.

A therapeutically effective amount preferably diminishes at least one symptom or effect associated with the N-6 substituted 7-deazapurine responsive state or condition being treated by at least about 20%, (more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80%) relative to untreated subjects. Assays can be designed by one skilled in the art to measure the diminishment of such symptoms and/or effects. Any art recognized assay capable of measuring such parameters are intended to be included as part of this invention. For example, if asthma is the state being treated, then the volume of air expended from the lungs of a subject can be measured before and after treatment for measurement of increase in the volume using an art recognized technique. Likewise, if inflammation is the state being treated, then the

area which is inflamed can be measured before and after treatment for measurement of diminishment in the area inflamed using an art recognized technique.

The term "cell" includes both prokaryotic and eukaryotic cells.

The term "animal" includes any organism with adenosine receptors or any organism susceptible to a N-6-substituted 7-deazapurine responsive state. Examples of animals include yeast, mammals, reptiles, and birds. It also includes transgenic animals.

The term "mammal" is art recognized and is intended to include an animal, more preferably a warm-blooded animal, most preferably cattle, sheep, pigs, horses, dogs, cats, rats, mice, and humans. Mammals susceptible to a N-6 substituted 7-deazapurine responsive state, inflammation, emphysema, asthma, central nervous system conditions, or acute respiratory distress syndrome, for example, are included as part of this invention.

In another aspect, the present invention pertains to methods for modulating an adenosine receptor(s) in a mammal by administering to the mammal a therapeutically effective amount of a N-6 substituted 7-deazapurine, such that modulation of the adenosine receptor in the mammal occurs. Suitable adenosine receptors include the families of A_1, A_2, or A_3. In a preferred embodiment, the N-6 substituted 7-deazapurine is an adenosine receptor antagonist.

The language "modulating an adenosine receptor" is intended to include those instances where a compound interacts with an adenosine receptor(s), causing increased, decreased or abnormal physiological activity associated with an adenosine

receptor or subsequent cascade effects resulting from the modulation of the adenosine receptor. Physiological activities associated with adenosine receptors include induction of sedation, vasodilation, suppression of cardiac rate and contractility, inhibition of platelet aggregbility, stimulation of gluconeogenesis, inhibition of lipolysis, opening of potassium channels, reducing flux of calcium channels, etc.

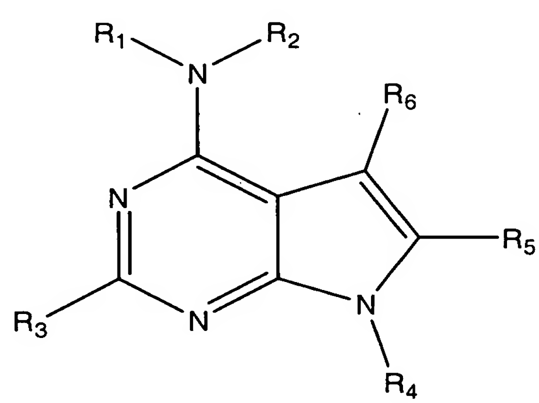
The terms "modulate", "modulating" and "modulation" are intended to include preventing, eradicating, or inhibiting the resulting increase of undesired physiological activity associated with abnormal stimulation of an adenosine receptor, *e.g.*, in the context of the therapeutic methods of the invention. In another embodiment, the term modulate includes antagonistic effects, *e.g.*, diminishment of the activity or production of mediators of allergy and allergic inflammation which results from the overstimulation of adenosine receptor(s). For example, the therapeutic deazapurines of the invention can interact with an adenosine receptor to inhibit, for example, adenylate cyclase activity.

The language "condition characterized by aberrant adenosine receptor activity" is intended to include those diseases, disorders or conditions which are associated with aberrant stimulation of an adenosine receptor, in that the stimulation of the receptor causes a biochemical and or physiological chain of events that is directly or indirectly associated with the disease, disorder or condition. This stimulation of an adenosine receptor does not have to be the sole causative agent of the disease, disorder or condition but merely be responsible for causing some of the symptoms typically associated with the disease, disorder, or condition being treated. The aberrant stimulation of the receptor can be the sole factor or at least one other agent can be involved in the

state being treated. Examples of conditions include those disease states listed *supra*, including inflammation, gastrointestinal disorders and those symptoms manifested by the presence of increased adenosine receptor activity. Preferred examples include those symptoms associated with asthma, allergic rhinitis, chronic obstructive pulmonary disease, emphysema, bronchitis, gastrointestinal disorders and glaucoma.

The language "treating or treatment of a condition characterized by aberrant adenosine receptor activity" is intended to include the alleviation of or diminishment of at least one symptom typically associated with the condition. The treatment also includes alleviation or diminishment of more than one symptom. Preferably, the treatment cures, *e.g.*, substantially eliminates, the symptoms associated with the condition.

The present invention pertains to compounds, N-6 substituted 7-deazapurines, having the formula I:



(I)

wherein R_1 and R_2 are each independently a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or together form a substituted or unsubstituted heterocyclic ring;

R_3 is a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety;

R_4 is a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety.

R_5 and R_6 are each independently a halogen atom, *e.g.*, chlorine, fluorine, or bromine, a hydrogen atom or a substituted or unsubstituted alkyl, aryl, or alkylaryl moiety or R_4 and R_5 or R_5 and R_6 together form a substituted or unsubstituted heterocyclic or carbocyclic ring. Also included, are pharmaceutically acceptable salts of the N-6 substituted 7-deazapurines.

In certain embodiments, R_1 and R_2 can each independently be a substituted or unsubstituted cycloalkyl or heteroarylalkyl moieties. In other embodiments, R_3 is a hydrogen atom or a substituted or unsubstituted heteroaryl moiety. In still other embodiments, R_4, R_5 and R_6 can each be independently a heteroaryl moiety.

In one embodiment, R_1 is a hydrogen atom, R_2 is a substituted or unsubstituted cyclohexane, cyclopentyl, cyclobutyl or cyclopropane moiety, R_3 is a substituted or unsubstituted phenyl moiety, R_4 is a hydrogen atom and R_5 and R_6 are both methyl groups.

In another embodiment, R_2 is a cyclohexanol, a cyclohexanediol, a cyclohexylsulfonamide, a cyclohexanamide, a cyclohexylester, a cyclohexene, a cyclopentanol or a cyclopentanediol and R_3 is a phenyl moiety.

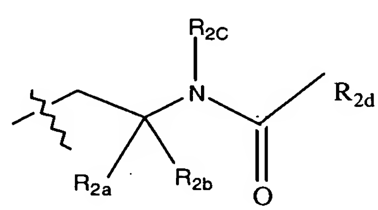
In still another embodiment, R_1 is a hydrogen atom, R_2 is a cyclohexanol, R_3 is a substituted or unsubstituted phenyl, pyridine, furan, cyclopentane, or thiophene moiety, R_4 is a hydrogen atom, a substituted alkyl, aryl or arylalkyl moiety, and R_5 and R_6 are each independently a hydrogen atom, or a

substituted or unsubstituted alkyl, aryl, or alkylaryl moiety.

In yet another embodiment, R_1 is a hydrogen atom, R_2 is substituted or unsubstituted alkylamine, arylamine, or alkylarylamine, a substituted or unsubstituted alkylamide, arylamide or alkylarylamide, a substituted or unsubstituted alkylsulfonamide, arylsulfonamide or alkylarylsulfonamide, a substituted or unsubstituted alkylurea, arylurea or alkylarylurea, a substituted or unsubstituted alkylcarbamate, arylcarbamate or alkylarylcarbamate, a substituted or unsubstituted alkylcarboxylic acid, arylcarboxylic acid or alkylarylcarboxylic acid, R_3 is a substituted or unsubstituted phenyl moiety, R_4 is a hydrogen atom and R_5 and R_6 are methyl groups.

In still another embodiment, R_2 is guanidine, a modified guanidine, cyanoguanidine, a thiourea, a thioamide or an amidine.

In one embodiment, R_2 can be



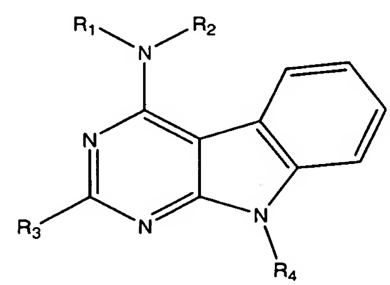
,

wherein R_{2a}-R_{2c} are each independently a hydrogen atom or a saturated or unsaturated alkyl, aryl or alkylaryl moiety and R_{2d} is a hydrogen atom or a saturated or unsaturated alkyl, aryl, or alkylaryl moiety, $NR_{2e}R_{2f}$, or OR_{2g}, wherein R_{2e}-R_{2g} are each independently a hydrogen atom or a saturated or unsaturated alkyl, aryl or alkylaryl moieties. Alternatively, R_{2a} and R_{2b} together can form a carbocyclic or heterocyclic ring having a ring size between about 3 and 8 members, *e.g.*, cyclopropyl, cyclopentyl, cyclohexyl groups.

In one aspect of the invention, both R_5 and R_6 are not methyl groups, preferably, one of R_5 and R_6 is an alkyl group, *e.g.*, a methyl group, and the other is a hydrogen atom.

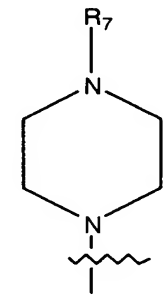
In another aspect of the invention, when R_4 is 1-phenylethyl and R_1 is a hydrogen atom, then R_3 is not phenyl, 2-chlorophenyl, 3-chlorophenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 3-methoxyphenyl or 4-methoxyphenyl or when R_4 and R_1 are 1-phenylethyl, then R_3 is not a hydrogen atom or when R_4 is a hydrogen atom and R_3 is a phenyl, then R_1 is not phenylethyl.

In another aspect of the invention, when R_5 and R_6 together form a carbocyclic ring, *e.g.*,



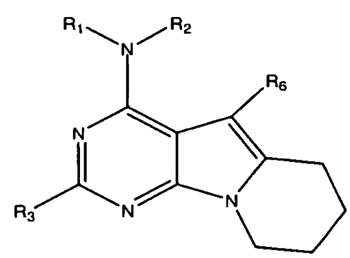
or pyrimido[4,5-6]indole, then R_3 is not phenyl when R_4 is 1-(4-methylphenyl)ethyl, phenylisopropyl, phenyl or 1-phenylethyl or when R_3 is not a hydrogen atom when R_4 is 1-phenylethyl. The carbocyclic ring formed by R_5 and R_6 can be either aromatic or aliphatic and can have between 4 and 12 carbon atoms, *e.g.*, naphthyl, phenylcyclohexyl, etc., preferably between 5 and 7 carbon atoms, *e.g.*, cyclopentyl or cyclohexyl. Alternatively, R_5 and R_6 together can form a heterocyclic ring, such as those disclosed below. Typical heterocyclic rings include between 4 and 12 carbon atoms, preferably between 5 and 7 carbon atoms, and can be either aromatic or aliphatic. The heterocyclic ring can be further substituted, including substitution of one or more carbon atoms of the ring structure with one or more heteroatoms.

In still another aspect of the invention, R_1 and R_2 form a heterocyclic ring. Representative examples include, but are not limited to, those heterocyclic rings listed below, such as morpholino, piperazine and the like, *e.g.*, 4-hydroxypiperidines, 4-aminopiperidines. Where R_1 and R_2 together form a piperazino group,



wherein R_7 can be a hydrogen atom or a substituted or unsubstituted alkyl, aryl or alkylaryl moiety.

In yet another aspect of the invention R_4 and R_5 together can form a heterocyclic ring, *e.g.*,



wherein the heterocyclic ring can be either aromatic or aliphatic and can form a ring having between 4 and 12 carbon atoms, *e.g.*, naphthyl, phenylcyclohexyl, etc. and can be either aromatic or aliphatic, *e.g.*, cyclohexyl, cyclopentyl. The heterocyclic ring can be further substituted, including substitution of carbon atoms of the ring structure with one or more heteroatoms. Alternatively, R_4 and R_5 together can form a heterocyclic ring, such as those disclosed below.

In certain embodiments, the N-6 substituted 7-deazapurine is not N-6 benzyl or N-6 phenylethyl substituted. In other

embodiments, R_4 is not benzyl or phenylethyl substituted. In preferred embodiments, R_1 and R_2 are both not hydrogen atoms. In still other preferred embodiments, R_3 is not H.

The compounds of the invention may comprise water-soluble prodrugs which are described in WO 99/33815, International Application No. PCT/US98/04595, filed March 9, 1998 and published July 8, 1999. The entire content of WO 99/33815 is expressly incorporated herein by reference. The water-soluble prodrugs are metabolized *in vivo* to an active drug, *e.g.*, by esterase catalyzed hydrolysis. Examples of potential prodrugs include deazapurines with, for example, R_2 as cycloalkyl substituted with -OC(O)(Z)NH_2, wherein Z is a side chain of a naturally or unnaturally occurring amino acid, or analog thereof, an α, β, γ, or ω amino acids, or a dipeptide. Preferred amino acid side chains include those of glycine, alanine, valine, leucine, isoleucine, lysine, α-methylalanine, aminocyclopropane carboxylic acid, azetidine-2-carboxylic acid, β-alanine, γ-aminobutyric acid, alanine-alanine, or glycine-alanine.

In a further embodiment, the invention features deazapurines of the formula (**I**), wherein R_1 is hydrogen; R_2 is substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkyl, or R_1 and R_2 together form a substituted or unsubstituted heterocyclic ring; R_3 is unsubstituted or substituted aryl; R_4 is hydrogen; and R_5 and R_6 are each independently hydrogen or alkyl, and pharmaceutically acceptable salts thereof. The deazapurines of this embodiment may potentially be selective A_3 receptor antagonists.

In one embodiment, R_2 is substituted (*e.g.*, hydroxy substituted) or unsubstituted cycloalkyl. In an advantageous subembodiment, R_1 and R_4 are hydrogen, R_3 is unsubstituted or substituted phenyl, and R_5 and R_6 are each alkyl. Preferably

R_2 is mono-hydroxycyclopentyl or mono-hydroxycyclohexyl. R_2 also may be substituted with -NH-C(=O)E, wherein E is substituted or unsubstituted C_1-C_4 alkyl (*e.g.*, alkylamine, *e.g.*, ethylamine.).

R_1 and R_2 may also together form a substituted or unsubstituted heterocyclic ring, which may be substituted with an amine or acetamido group.

In another aspect, R_2 may be -A-NHC(=O)B, wherein A is unsubstituted C_1-C_4 alkyl (*e.g.*, ethyl, propyl, butyl), and B is substituted or unsubstituted C_1-C_4 alkyl (*e.g.*, methyl, aminoalkyl, e.g., aminomethyl or aminoethyl, alkylamino, e.g., methylamino, ethylamino), preferably when R_1 and R_4 are hydrogen, R_3 is unsubstituted or substituted phenyl, and R_5 and R_6 are each alkyl. B may be substituted or unsubstituted cycloalkyl, e.g., cyclopropyl or 1-amino-cyclopropyl.

In another embodiment, R_3 may be substituted or unsubstituted phenyl, preferably when R_5 and R_6 are each alkyl. Preferably, R_3 may have one or more substituents (e.g., *o*-, *m*- or *p*-chlorophenyl, *o*-, *m*- or *p*- fluorophenyl).

Advantageously, R_3 may be substituted or unsubstituted heteroaryl, preferably when R_5 and R_6 are each alkyl. Examples of heteroaryl groups include pyridyl, pyrimidyl, pyridazinyl, pyrazinyl, pyrrolyl, triazolyl, thioazolyl, oxazolyl, oxadiazolyl, furanyl, methylenedioxyphenyl and thiophenyl. Preferably, R_3 is 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl or 3- pyrimidyl.

Preferably in one embodiment, R_5 and R_6 are each hydrogen. In another, R_5 and R_6 are each methyl.

In a particularly preferred embodiment, the deazapurines of

the invention are water-soluble prodrugs that can be metabolized *in vivo* to an active drug, e.g. by esterase catalyzed hydrolysis. Preferably the prodrug comprises an R_2 group which is cycloalkyl substituted with $-OC(O)(Z)NH_2$, wherein Z is a side chain of a naturally or unnaturally occurring amino acid, an analog thereof, an α, β, γ, or ω amino acid, or a dipeptide. Examples of preferred side chains include the side chains of glycine, alanine, valine, leucine, isoleucine, lysine, α-methylalanine, aminocyclopropane carboxylic acid, azetidine-2-carboxylic acid, β-alanine, γ-aminobutyric acid, alanine-alanine, or glycine-alanine.

In a particularly preferred embodiment, Z is a side chain of glycine, R_2 is cyclohexyl, R_3 is phenyl, and R_5 and R_6 are methyl.

In another embodiment, the deazapurine is 4-(*cis*-3-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo [2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(*cis*-3-(2-aminoacetoxy) cyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine trifluoroacetic acid salt.

In another embodiment, the deazapurine is 4-(3-acetamido)piperidinyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine.

In another embodiment, the deazapurine is 4-(2-N'-methylureapropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine.

In another embodiment, the deazapurine is 4-(2-acetamidobutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine.

In another embodiment, the deazapurine is 4-(2-N'-methylureabutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine.

In another embodiment, the deazapurine is 4-(2-aminocyclopropylacetamidoethyl)amino-2-phenyl-7*H*-pyrrolo [2,3d]pyrimidine.

In another embodiment, the deazapurine is 4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-chlorophenyl)-7*H*-pyrrolo[2,3d] pyrimidine.

In another embodiment, the deazapurine is 4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d] pyrimidine.

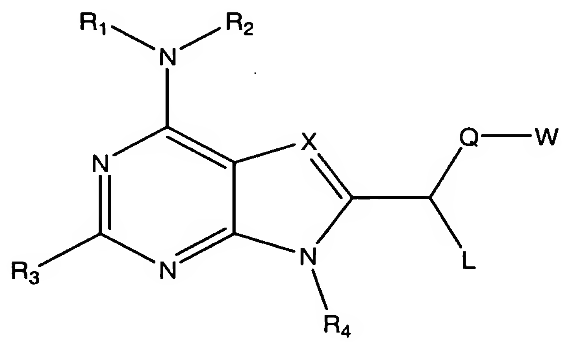
In another embodiment, the deazapurine is 4-(*trans*-4-hydroxycyclohexyl)amino-2-(4-pyridyl)-7*H*-pyrrolo[2,3d] pyrimidine.

In yet another embodiment, the invention features a method for inhibiting the activity of an adenosine receptor (*e.g.*, A_1, A_{2A}, A_{2B}, or, preferably, A_3) in a cell, by contacting the cell with N-6 substituted 7-deazapurine (*e.g.*, preferably, an adenosine receptor antagonist).

In another aspect, the invention features a method for treating damage to the eye of an animal(*e.g.*, a human) by administering to the animal an effective amount of an N-6 substituted 7-deazapurine. Preferably, the N-6 substituted 7-deazapurine is an antagonist of A_3 adenosine receptors in cells of the animal. The damage is to the retina or the optic nerve head and may be acute or chronic. The damage may be the result of, for example, glaucoma, edema, ischemia, hypoxia or

trauma.

In a preferred embodiment, the invention features a deazapurine having the formula II:



(II)

wherein X is N or CR_6;

R_1 and R_2 are each independently hydrogen, or substituted or unsubstituted alkoxy, aminoalkyl, alkyl, aryl, or alkylaryl, or together form a substituted or unsubstituted heterocyclic ring, provided that both R_1 and R_2 are both not hydrogen;

R_3 is substituted or unsubstituted alkyl, arylalkyl, or aryl;

R_4 is hydrogen or substituted or unsubstituted C_1-C_6 alkyl;

L is hydrogen, substituted or unsubstituted alkyl, or R_4 and L together form a substituted or unsubstituted heterocyclic or carbocyclic ring;

R_6 is hydrogen, substituted or unsubstituted alkyl, or halogen;

Q is CH_2, O, S, or $NR_{7'}$, wherein $R_{7'}$ is hydrogen or substituted or unsubstituted C_1-C_6 alkyl; and

W is unsubstituted or substituted alkyl, cycloalkyl, alkynyl, aryl, arylalkyl, biaryl, heteroaryl, substituted carbonyl, substituted thiocarbonyl, or substituted sulfonyl, provided that if R_3 is pyrrolidino, then R_4 is not methyl.

In one embodiment, in compounds of formula II, X is CR_6 and Q is CH_2, O, S, or NH. In another embodiment, X is N.

In a further embodiment of compounds of formula II, W is substituted or unsubstituted aryl, 5- or 6- member heteroaryl, or biaryl. W may be substituted with one or more substituents. Examples of substituents include: halogen, hydroxy, alkoxy, amino, aminoalkyl, aminocarboxyamide, CN, CF_3, CO_2R_8, $CONHR_8$, $CONR_8R_9$, SOR_8, SO_2R_8, and $SO_2NR_8R_9$, wherein R_8 and R_9 are each independently hydrogen, or substituted or unsubstituted alkyl, cycloalkyl, aryl, or arylalkyl. Preferably, W may be substituted or unsubstituted phenyl, *e.g.*, methylenedioxyphenyl. W also may be a substituted or unsubstituted 5-membered heteroaryl ring, *e.g.*, pyrrole, pyrazole, oxazole, imidazole, triazole, tetrazole, furan, thiophene, thiazole, and oxadiazole. Preferably, W may be a 6-member heteroaryl ring, *e.g.*, pyridyl, pyrimidyl, pyridazinyl, pyrazinyl, and thiophenyl. In a preferred embodiment, W is 2-pyridyl, 3- pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, or 5-pyrimidyl.

In one advantageous embodiment of compounds of formula II, Q is NH and W is a 3-pyrazolo ring which is unsubstituted or N-substituted by substituted or unsubstituted alkyl, cycloalkyl, aryl, or arylalkyl.

In another embodiment of compounds of formula II, Q is oxygen, and W is a 2-thiazolo ring which is unsubstituted or substituted by substituted or unsubstituted alkyl, cycloalkyl, aryl, or arylalkyl.

In another embodiment of compounds of formula II, W is substituted or unsubstituted alkyl, cycloalkyl *e.g.*, cyclopentyl, or arylalkyl. Examples of substituents include halogen, hydroxy, substituted or unsubstituted alkyl,

cycloalkyl, aryl, arylalkyl, or NHR_{10}, wherein R_{10} is hydrogen, or substituted or unsubstituted alkyl, cycloalkyl, aryl, or arylalkyl.

In yet another embodiment, the invention features a deazapurine of formula II wherein W is $-(CH_2)_a-C(=O)Y$ or $-(CH_2)_a-C(=S)Y$, and a is an integer from 0 to 3, Y is aryl, alkyl, arylalkyl, cycloalkyl, heteroaryl, alkynyl, $NHR_{11}R_{12}$, or, provided that Q is NH, OR_{13}, wherein R_{11}, R_{12} and R_{13} are each independently hydrogen, or unsubstituted or substituted alkyl, aryl, arylalkyl, or cycloalkyl. Preferably, Y is a 5- or 6- member heteroaryl ring.

Furthermore, W may be $-(CH_2)_b-S(=O)_j Y$, wherein j is 1 or 2, b is 0, 1, 2, or 3, Y is aryl, alkyl, arylalkyl, cycloalkyl, alkynyl, heteroaryl, $NHR_{14}R_{15}$, provided that when b is 1, Q is CH_2, OR_{16}, and wherein R_{14}, R_{15}, and R_{16} are each independently hydrogen, or unsubstituted or substituted alkyl, aryl, arylalkyl, or cycloalkyl.

In another embodiment, R_3 is selected from the group consisting of substituted and unsubstituted phenyl, pyridyl, pyrimidyl, pyridazinyl, pyrazinal, pyrrolyl, triazolyl, thioazolyl, oxazolyl, oxadiazolyl, pyrazolyl, furanyl, methylenedioxyphenyl, and thiophenyl. When R_3 is phenyl, it may be substituted with, for example, hydroxyl, alkoxy (*e.g.*, methoxy), alkyl (*e.g.*, tolyl), and halogen, (*e.g.*, *o*-, *m*-, or *p*- fluorophenyl or *o*-, *m*-, or *p*- chlorophenyl). Advantageously, R_3 may be 2-, 3-, or 4- pyridyl or 2- or 3- pyrimidyl.

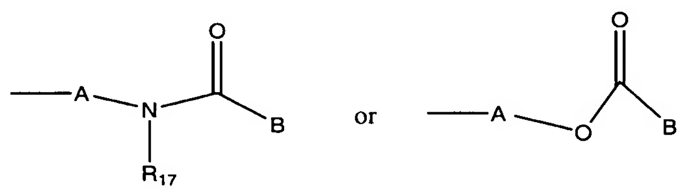
The invention also pertains to a deazapurine wherein R_6 is hydrogen or C_1-C_3 alkyl. Preferably, R_6 is hydrogen.

The invention also includes deazapurines wherein R_1 is

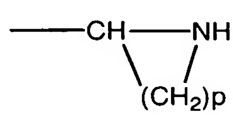
hydrogen, and R_2 is substituted or unsubstituted alkyl or alkoxy, substituted or unsubstituted alkylamine, arylamine, or alkylarylamine, substituted or unsubstituted aminoalkyl, amino aryl, or aminoalkylaryl, substituted or unsubstituted alkylamide, arylamide or alkylarylamide, substituted or unsubstituted alkylsulfonamide, arylsulfonamide or alkylarylsulfonamide, substituted or unsubstituted alkylurea, arylurea or alkylarylurea, substituted or unsubstituted alkylcarbamate, arylcarbamate or alkylarylcarbamate, or substituted or unsubstituted alkylcarboxylic acid, arylcarboxylic acid or alkylarylcarboxylic acid.

Preferably, R_2 is substituted or unsubstituted cycloalkyl, *e.g.*, mono- or dihydroxy-substituted cyclohexyl or cyclopentyl (preferably, monohydroxy-substituted cyclohexyl or monohydroxy-substituted cyclopentyl).

Advantageously, R_2 may be of the following formula:



wherein A is C_1-C_6 alkyl, C_3-C_7 cycloalkyl, a chain of one to seven atoms, or a ring of three to seven atoms, optionally substituted with C_1-C_6 alkyl, halogens, hydroxyl, carboxyl, thiol, or amino groups; wherein B is methyl, $N(Me)_2$, $N(Et)_2$, NHMe, NHEt, $(CH_2)_rNH_3+$, $NH(CH_2)_rCH_3$, $(CH_2)_rNH_2$, $(CH_2)_rCH(CH_3)NH_2$, $(CH_2)_rNHMe$, $(CH_2)_rOH$, CH_2CN, $(CH_2)_mCO_2H$, $CHR_{18}R_{19}$, or CHMeOH, wherein r is an integer from 0 to 2, m is 1 or 2, R_{18} is alkyl, R_{19} is NH_3+ or CO_2H or R_{18} and R_{19} together are:



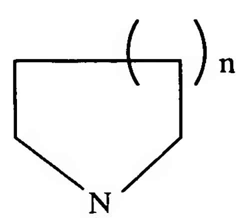
wherein p is 2 or 3; and R_{17} is C_1-C_6 alkyl, C_3-C_7 cycloalkyl, a chain of one to seven atoms, or a ring of three to seven atoms, optionally substituted with C_1-C_6 alkyl, halogens, hydroxyl, carboxyl, thiol, or amino groups.

Advantageously, A is unsubstituted or substituted C_1-C_6 alkyl. B may be substituted or unsubstituted C_1-C_6 alkyl.

In a preferred embodiment, R_2 is of the formula -A-NHC(=O)B. In a particularly advantageous embodiment, A is $-CH_2CH_2-$ and B is methyl.

The compounds of the invention may comprise water-soluble prodrugs which are metabolized *in vivo* to an active drug, *e.g.*, by esterase catalyzed hydrolysis. Examples of potential prodrugs include deazapurines with, for example, R_2 as cycloalkyl substituted with $-OC(O)(Z)NH_2$, wherein Z is a side chain of a naturally or unnaturally occurring amino acid, or analog thereof, an α, β, γ, or ω amino acid, or a dipeptide. Preferred amino acid side chains include those of glycine, alanine, valine, leucine, isoleucine, lysine, α-methylalanine, aminocyclopropane carboxylic acid, azetidine-2-carboxylic acid, β-alanine, γ-aminobutyric acid, alanine-alanine, or glycine-alanine.

In another embodiment, R_1 and R_2 together are:



wherein n is 1 or 2, and wherein the ring may be optionally substituted with one or more hydroxyl, amino, thiol, carboxyl, halogen, CH_2OH, $CH_2NHC(=O)$alkyl, or $CH_2NHC(=O)NH$alkyl groups.

Preferably, n is 1 or 2 and said ring is substituted with -NHC(=O)alkyl.

In one advantageous embodiment, R_1 is hydrogen, R_2 is substituted or unsubstituted C_1-C_6 alkyl, R_3 is substituted or unsubstituted phenyl, R_4 is hydrogen, L is hydrogen or substituted or unsubstituted C_1-C_6 alkyl, Q is O, S or $NR_{7'}$, wherein $R_{7'}$ is hydrogen or substituted or unsubstituted C_1-C_6 alkyl, and W is substituted or unsubstituted aryl. Preferably, R_2 is -A-NHC(=O)B, wherein A and B are each independently unsubstituted or substituted C_1-C_4 alkyl. For example, A may be CH_2CH_2. B may be, for example, alkyl (*e.g.*, methyl), or aminoalkyl (*e.g.*, aminomethyl). Preferably, R_3 is unsubstituted phenyl and L is hydrogen. R_6 may be methyl or preferably, hydrogen. Preferably, Q is O, S, or $NR_{7'}$ wherein $R_{7'}$ is hydrogen or substituted or unsubstituted C_1-C_6 alkyl, *e.g.*, methyl. W is unsubstituted or substituted phenyl (*e.g.*, alkoxy, halogen substituted). Preferably, W is *p*-fluorophenyl, *p*-chlorophenyl, or *p*-methoxyphenyl. W may also be heteroaryl, *e.g.*, 2-pyridyl.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(4-fluorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(4-chlorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(4-methoxyphenoxy)methyl-2-

phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(2-pyridyloxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-acetylaminoethyl) amino-6-(N-methyl-N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

In a particularly preferred embodiment, the deazapurine is 4-(2-N'-methylureaethyl) amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

The invention further pertains to a method for inhibiting the activity of an adenosine receptor (*e.g.*, an A_{2b} adenosine receptor) in a cell by contacting the cell with a compound of the invention. Preferably, the compound is an antagonist of the receptor.

The invention also pertains to a method for treating a gastrointestinal disorder (*e.g.*, diarrhea) in an animal by administering to an animal an effective amount of a compound of the invention (*e.g.*, an antagonist of A_{2b}). Preferably, the animal is a human.

In another embodiment, the invention relates to a pharmaceutical composition containing an N-6 substituted 7-deazapurine of the invention and a pharmaceutically acceptable carrier.

The invention also pertains to a method for treating a N-6 substituted 7-deazapurine responsive state in an animal, by administering to a mammal a therapeutically effective amount of a deazapurine of the invention, such that treatment of a N-6 substituted 7-deazapurine responsive state in the animal occurs. Advantageously, the disease state may be a disorder mediated by adenosine. Examples of preferred disease states include: central nervous system disorders, cardiovascular disorders, renal disorders, inflammatory disorders, allergic disorders, gastrointestinal disorders, eye disorders, and respiratory disorders.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen, sulfur or phosphorous atoms. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term "alkyl" as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate,

alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, *e.g.*, with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (*e.g.*, phenylmethyl (benzyl)). The term "alkyl" also includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "aryl" as used herein, refers to the radical of aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, tetrazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate,

phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.*, tetralin).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively. For example, the invention contemplates cyano and propargyl groups.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure, even more preferably one to three carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

The terms "alkoxyalkyl", "polyaminoalkyl" and "thioalkoxyalkyl" refer to alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen or sulfur atoms.

The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings,

e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The term "amino acids" includes naturally and unnaturally occurring amino acids found in proteins such as glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan. Amino acid analogs include amino acids with lengthened or shortened side chains or variant side chains with appropriate functional groups. Amino acids also include D and L stereoisomers of an amino acid when the structure of the amino acid admits of stereoisomeric forms. The term "dipeptide" includes two or more amino acids linked together. Preferably, dipeptides are two amino acids linked via a peptide linkage. Particularly preferred dipeptides include, for example, alanine-alanine and

glycine-alanine.

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms and thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. All such isomeric forms of these compounds are expressly included in this invention. Each stereogenic carbon may be of the R or S configuration. It is to be understood accordingly that the isomers arising from such asymmetry (*e.g.*, all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis.

The invention further pertains to pharmaceutical compositions for treating a N-6 substituted 7-deazapurine responsive state in a mammal, *e.g.*, respiratory disorders (*e.g.*, asthma, bronchitis, chronic obstructive pulmonary disorder, and allergic rhinitis), renal disorders, gastrointestinal disorders, and eye disorders. The pharmaceutical composition includes a therapeutically effective amount of a N-6 substituted 7-deazapurine, described *supra*, and a pharmaceutically acceptable carrier. It is to be understood, that all of the deazapurines described above are included for therapeutic treatment. It is to be further understood that the deazapurines of the invention can be used alone or in combination with other deazapurines of the invention or in combination with additional therapeutic compounds, such as antibiotics, antiinflammatories, or anticancer agents, for example.

The term "antibiotic" is art recognized and is intended to include those substances produced by growing microorganisms and synthetic derivatives thereof, which eliminate or inhibit

growth of pathogens and are selectively toxic to the pathogen while producing minimal or no deleterious effects upon the infected host subject. Suitable examples of antibiotics include, but are not limited to, the principle classes of aminoglycosides, cephalosporins, chloramphenicols, fuscidic acids, macrolides, penicillins, polymixins, tetracyclines and streptomycins.

The term "antiinflammatory" is art recognized and is intended to include those agents which act on body mechanisms, without directly antagonizing the causative agent of the inflammation such as glucocorticoids, aspirin, ibuprofen, NSAIDS, etc.

The term "anticancer agent" is art recognized and is intended to include those agents which diminish, eradicate, or prevent growth of cancer cells without, preferably, adversely affecting other physiological functions. Representative examples include cisplatin and cyclophosphamide.

When the compounds of the present invention are administered as pharmaceuticals, to humans and mammals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it can perform its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must

be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain embodiments of the present compounds can contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate,

mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, *e.g.*, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters *via* treatment with an alcohol in the presence of a catalyst. Hydroxyl containing derivatives can be converted into esters *via* treatment with an esterifying agent such as alkanoyl halides. The term is further intended to include lower hydrocarbon

groups capable of being solvated under physiological conditions, *e.g.*, alkyl esters, methyl, ethyl and propyl esters. (See, for example, Berge *et al.*, *supra.*)

The invention further contemplates the use of prodrugs which are converted *in vivo* to the therapeutic compounds of the invention (see, *e.g.*, R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chapter 8). Such prodrugs can be used to alter the biodistribution (*e.g.*, to allow compounds which would not typically enter the reactive site of the protease) or the pharmacokinetics of the therapeutic compound. For example, a carboxylic acid group, can be esterified, *e.g.*, with a methyl group or an ethyl group to yield an ester. When the ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, reductively or hydrolytically, to reveal the anionic group. An anionic group can be esterified with moieties (*e.g.*, acyloxymethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound. In another embodiment, the prodrug is a reduced form of a sulfate or sulfonate, *e.g.*, a thiol, which is oxidized *in vivo* to the therapeutic compound. Furthermore, an anionic moiety can be esterified to a group which is actively transported *in vivo*, or which is selectively taken up by target organs. The ester can be selected to allow specific targeting of the therapeutic moieties to particular reactive sites, as described below for carrier moieties.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets,

lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds

of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert dilutents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert dilutents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for

vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention. Preferably, the pharmaceutical preparation is an ophthalmic formulation (e.g., an periocular, retrobulbar or intraocular injection formulation, a systemic formulation, or a surgical irrigating solution).
The ophthalmic formulations of the present invention may include one or more deazapurines and a pharmaceutically acceptable vehicle. Various types of vehicles may be used. The vehicles will generally be aqueous in nature. Aqueous solutions are generally preferred, based on case of formulation, as well as a patient's ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the deazapurines of the present invention may also be readily incorporated into other types of compositions, such as suspensions, viscous or semi-viscous gels or other types of solid or semi-solid compositions. The ophthalmic compositions of the present invention may also include various other ingredients, such as buffers, preservatives, co-solvents and viscosity building agents.

An appropriate buffer system (e.g., sodium phosphate, sodium acetate or sodium borate) may be added to prevent pH drift under storage conditions.

Ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% weight/volume ("% w/v").

When the deazapurines of the present invention are administered during intraocular surgical procedures, such as through retrobulbar or periocular injection and intraocular perfusion or injection, the use of balanced salt irrigating solutions as vehicles are most preferred. BSS® Sterile Irrigating Solution and BSS Plus® Sterile Intraocular Irrigating Solution (Alcon Laboratories, Inc., Fort Worth, Texas, USA) are examples of physiologically balanced intraocular irrigating solutions. The latter type of solution is described in U.S. Pat. No. 4,550,022 (Garabedian, *et al.*), the entire contents of which are hereby incorporated in the present specification by reference. Retrobulbar and periocular injections are known to those skilled in the art and are described in numerous publications including, for example, *Ophthalmic Surgery: Principles of Practice,* Ed., G. L. Spaeth. W. B. Sanders Co., Philadelphia, Pa., U.S.A., pages 85-87 (1990).

As indicated above, use of deazapurines to prevent or reduce damage to retinal and optic nerve head tissues at the cellular level is a particularly important aspect of one embodiment of the invention. Ophthalmic conditions which may be treated include, but are not limited to, retinopathies, macular degeneration, ocular ischemia, glaucoma, and damage associated with injuries to ophthalmic tissues, such as ischemia reperfusion injuries, photochemical injuries, and injuries associated with ocular surgery, particularly injuries to the retina or optic nerve head by exposure to light or surgical instruments. The compounds may also be used as an adjunct to ophthalmic surgery, such as by vitreal or subconjunctival injection following ophthalmic surgery. The compounds may be used for acute treatment of temporary conditions, or may be administered chronically, especially in the case of degenerative disease. The compounds may also be used prophylactically, especially prior to ocular surgery or

noninvasive ophthalmic procedures, or other types of surgery.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular,

intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systematically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of

factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 200 mg per kilogram of body weight per day, more preferably from about 0.01 to about 150 mg per kg per day, and still more preferably from about 0.2 to about 140 mg per kg per day.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

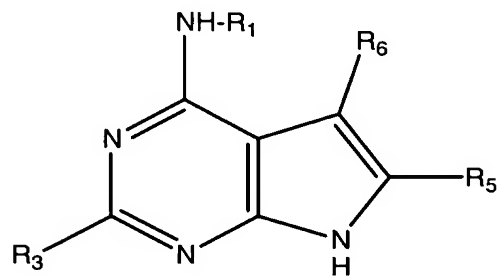
While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical composition.

The present invention also pertains to packaged pharmaceutical compositions for treating a N-6 substituted 7 deazapurine responsive state, *e.g.*, undesirable increased adenosine receptor activity in a mammal. The packaged pharmaceutical compositions include a container holding a therapeutically effective amount of at least one deazapurine as described *supra* and instructions for using the deazapurine for treating the deazapurine responsive state in the mammal.

The deazapurines of the invention can be prepared using standard methods for organic synthesis. Deazapurines can be purified by reverse phase HPLC, chromatography, recrystallization, etc. and their structures confirmed by mass spectral analysis, elemental analysis, IR and/or NMR spectroscopy.

Typically, synthesis of the intermediates as well as the deazapurines of the invention is performed in solution. The addition and removal of one or more protecting groups is alsotypical practice and is known to those skilled in the art. Typical synthetic schemes for the preparation of deazapurine intermediates of the invention are outlined below in Scheme I.

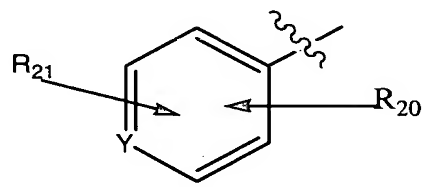
This invention further provides a compound having the structure (IV):



IV

wherein R_1 is *trans*-4-hydroxy cyclohexyl, 2-methylamino carbonylamino cyclohexyl, acetylamino ethyl, or methylamino carbonylamino ethyl;

wherein R_3 is a substituted or unsubstituted four to six membered ring, phenyl, pyrrole, thiophene, furan, thiazole, imidazole, pyrazole, 1,2,4-triazole, pyridine, 2(1H)-pyridone, 4(1H)-pyridone, pyrazine, pyrimidine, pyridazine, isothiazole, isoxazole, oxazole, tetrazole, naphthalene, tetralin, naphthyridine, benzofuran, benzothiophene, indole, 2,3-dihydroindole, 1H-indole, indoline, benzopyrazole, 1,3-benzodioxole, benzoxazole, purine, coumarin, chromone, quinoline, tetrahydroquinoline, isoquinoline, benzimidazole, quinazoline, pyrido[2,3-b]pyrazine, pyrido[3,4-b]pyrazine, pyrido[3,2-c]pyridazine, pyrido[3,4-b]-pyridine, 1H-pyrazole[3,4-d]pyrimidine, pteridine, 2(1H)-quinolone, 1(2H)-isoquinolone, 1,4-benzisoxazine, benzothiazole, quinoxaline, quinoline-N-oxide, isoquinoline-N-oxide, quinoxaline-N-oxide, quinazoline-N-oxide, benzoxazine, phthalazine, cinnoline, or having a structure:



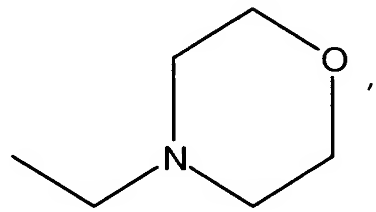
wherein Y is carbon or nitrogen;

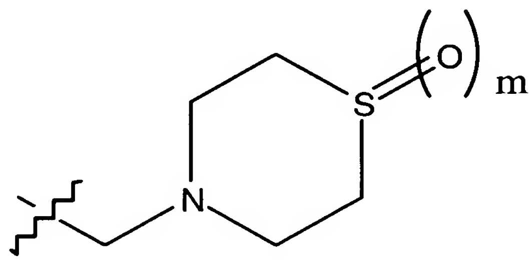
wherein R_{20} and R_{21} are independently H, substituted or unsubstituted alkyl, substituted or unsubstituted aryl, halogen, methoxy, methyl amino, or methyl thio;

wherein R_5 is H, alkyl, substituted alkyl, aryl, arylalkyl, amino, substituted aryl, wherein said substituted alkyl is $-C(R_7)(R_8)XR_{9'}$, wherein X is O, S, or $NR_{10'}$, wherein R_7 and R_8 are each independently H or alkyl, wherein $R_{9'}$ and $R_{10'}$ are each independently alkyl or cycloalkyl, or $NR_{9'}R_{10'}$ is a substituted or unsubstituted ring of between 4 and 7 members;

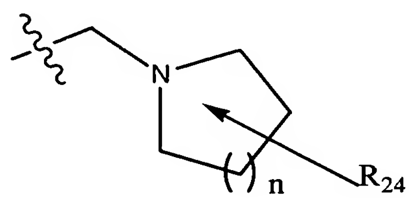
wherein R_6 is H, alkyl, substituted alkyl, cycloalkyl; or a pharmaceutically acceptable salt, a prodrug derivative, or a biologically active metabolite, with proviso that when R_1 acetylamino ethyl, R_3 is not 4-pyridyl.

In one embodiment of the compound having structure IV, $NR_{9'}R_{10'}$ is a substituted or unsubstituted ring of between 4 and 7 members which is selected from the group consisting of:

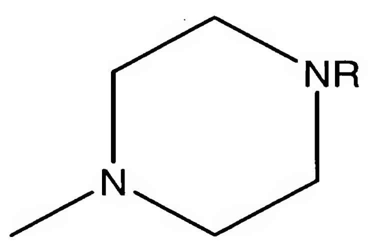




wherein m is 0, 1, or 2,

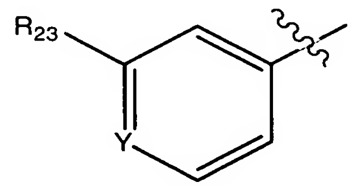


wherein n is 0, 1, 2, or 3; wherein R_{24} is hydrogen, -OH, $-CH_2OH$, $-C(=O)NR_9R_{10}$, NHR_{22}; wherein R_{22} is $-C(=O)CH_3$, or $-SO_2Me$, or



wherein R is H, alkyl, or aryl.

In another embodiment of the compound having structure IV, R_3 has the structure:



wherein Y is carbon or nitrogen; wherein R_{23} is H, or halogen, -O-alkyl group, amine group, or sulfide group;

wherein R_5 is H, alkyl, substituted alkyl, aryl, arylalkyl, amino, substituted aryl, wherein said substituted alkyl is $-C(R_7)(R_8)NR_{9'}R_{10'}$, wherein R_7 and R_8 are each independently H or alkyl, wherein $R_{9'}$ and $R_{10'}$ are each independently alkyl or cycloalkyl, or $R_{9'}$, $R_{10'}$ and the nitrogen together form a substituted or unsubstituted ring of between 4 and 7 members.

In another embodiment of the compound, Y is carbon.

In another embodiment of the compound, R_{23} is hydrogen.

In another embodiment of the compound, R_6 is hydrogen.

In another embodiment of the compound, R_5 is hydrogen.

In another embodiment of the compound, R_5 and R_6 are each methyl.

In another embodiment of the compound, R_5 is $-C(R_7)(R_8)NR_{9'}R_{10'}$, wherein R_7 and R_8 are each independently H or alkyl, wherein $R_{9'}$ and $R_{10'}$ are each independently alkyl or cycloalkyl, or $R_{9'}$, $R_{10'}$ and the nitrogen together form a substituted or unsubstituted ring of between 4 and 7 members.

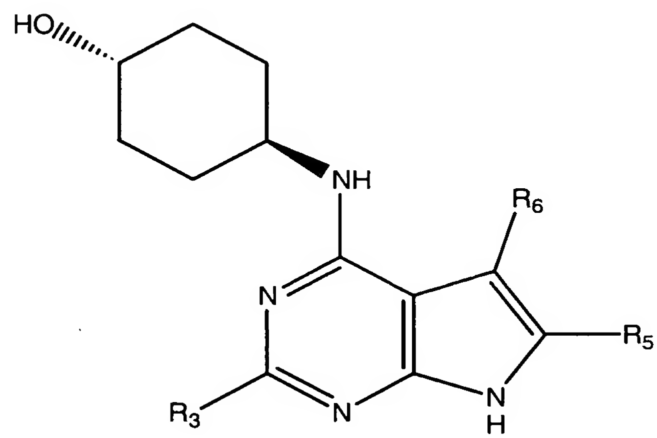
In another embodiment of the compound, R_{23} is halogen.

In another embodiment of the compound, Y is nitrogen.

In yet another embodiment of the compound, R_{23} is hydrogen.

In a further embodiment of the compound, R_5 and R_6 are each hydrogen.

This invention also provides a compound having the structure (V):



V

wherein R_3 is aryl, substituted aryl, or heteroaryl;

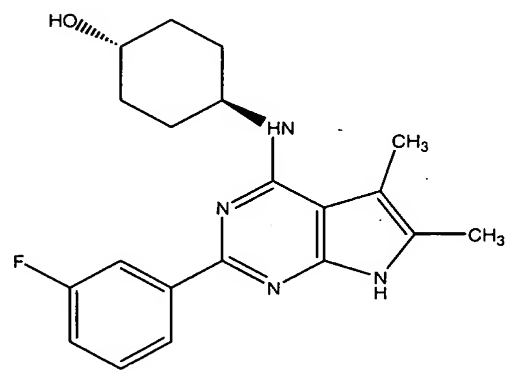
wherein R_5 is H, alkyl, substituted alkyl, aryl, arylalkyl, amino, substituted aryl, wherein said substituted alkyl is $-C(R_7)(R_8)NR_{9'}R_{10'}$, wherein R_7 and R_8 are each H or alkyl, wherein $R_{9'}$ and $R_{10'}$ are each alkyl or cycloalkyl, or $R_{9'}$, $R_{10'}$ and the nitrogen together form a ring system of between 4 and 7 members; and

wherein R_6 is H, alkyl, substituted alkyl, or cycloalkyl.

In one embodiment of the compound having structure V, R_7 and R_8 are each H; wherein $R_{9'}$ is H and $R_{10'}$ is $-R_{12}C(=O)R_{13}$.

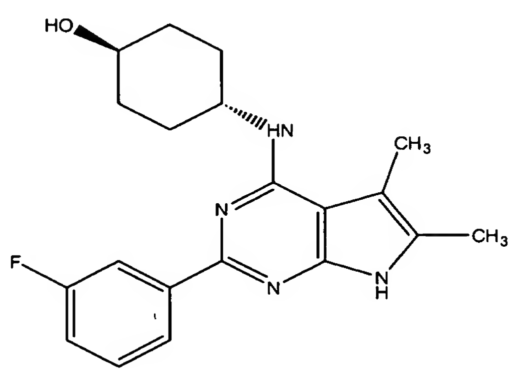
In another embodiment of the compound having structure V, R_7 and R_8 are each H; wherein the ring system is morpholino, thiomorpholino, N-4-substituted piperazino, 2-substituted piperazine, or R_{24} substituted pyrrolidino, piperidine, wherein R_{24} is H, OH, CH_2OH, $-C(=O)NR_9R_{10}$, NR_{22}, wherein R_{22} is $-C(=O)CH_3$, $-SO_2Me$.

In another embodiment of the compound, the compound has the following structure:

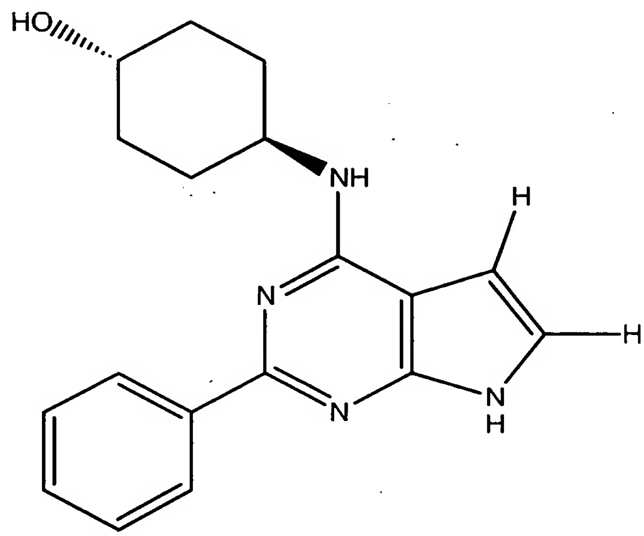


(Compound 706)

In another embodiment of the compound, the compound has the structure:

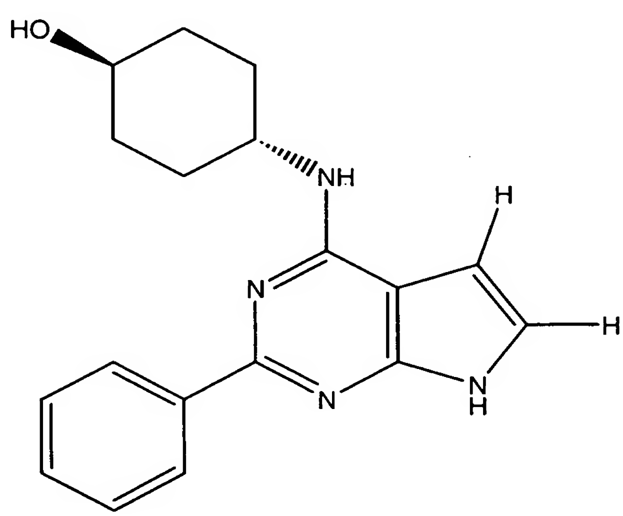


In another embodiment of the compound, the compound has the structure:



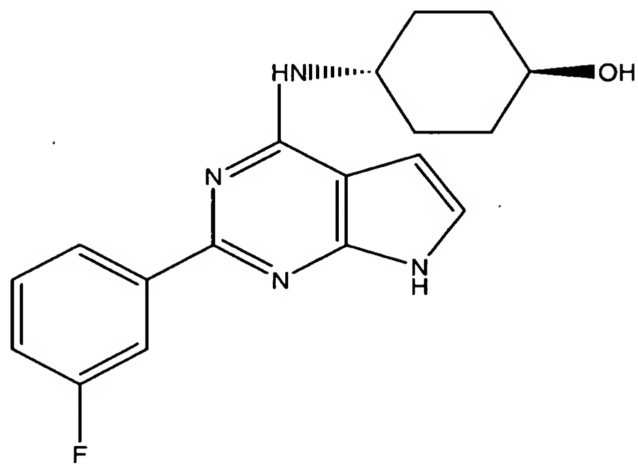
(Compound 1318-a)

In another embodiment of the compound, the compound has the structure:



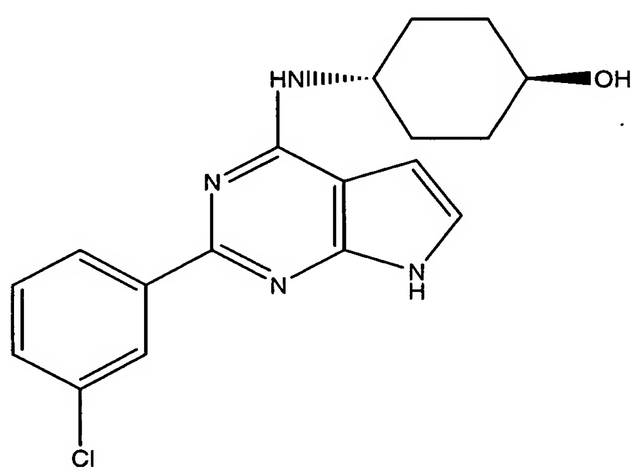
(Compound 1318-b)

In another embodiment of the compound, the compound has the structure:



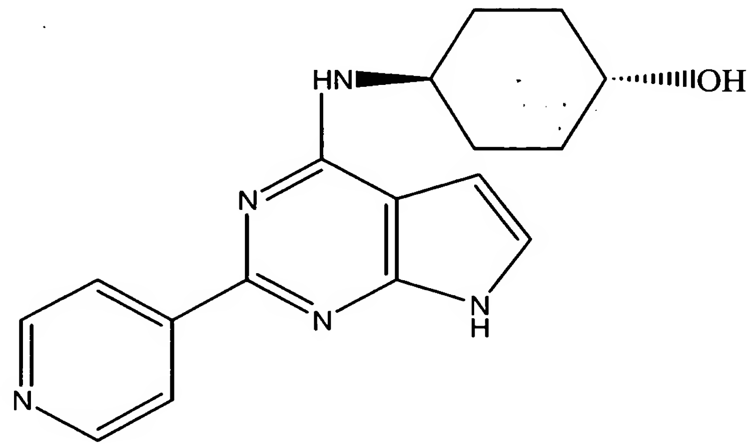
(Compound 1319)

In another embodiment of the compound, the compound has the structure:



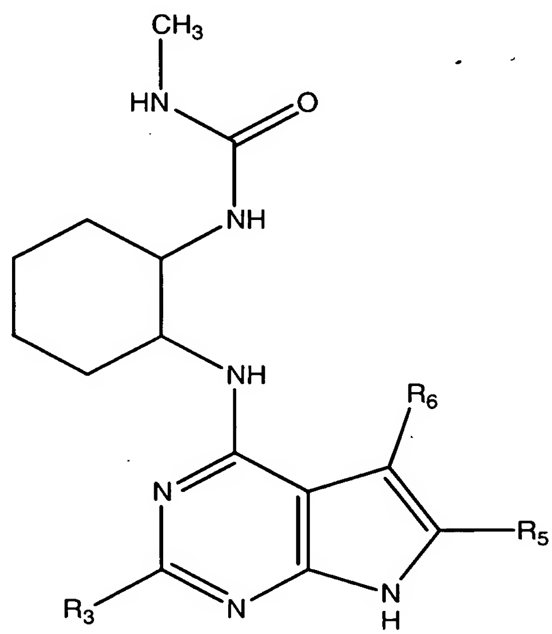
(Compound 1320)

In another embodiment of the compound, the compound has the structure:



(Compound 1321)

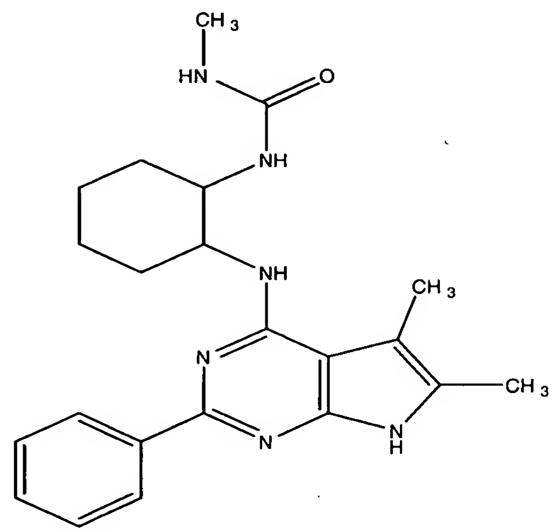
A compound having the structure:



VI

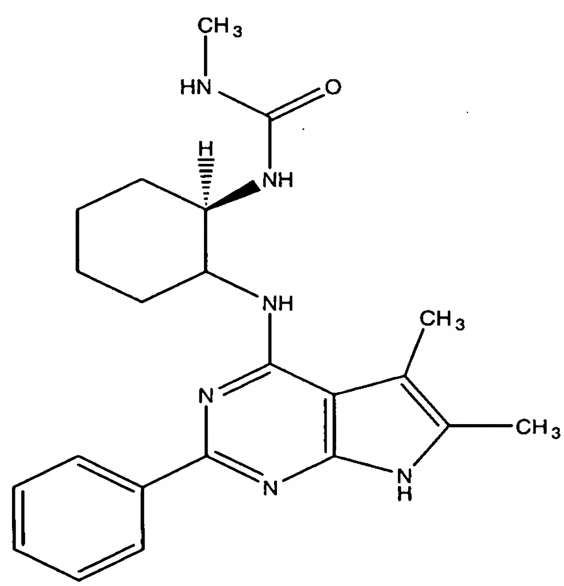
wherein R_3 is a 5-6 membered aromatic ring; wherein R_5 and R_6 are independently H, or alkyl.

In one embodiment of the compound, the compound has the structure:

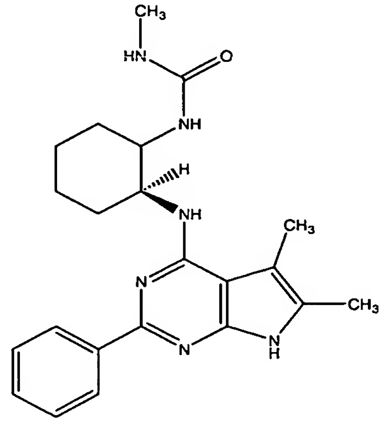


(Compound 1500)

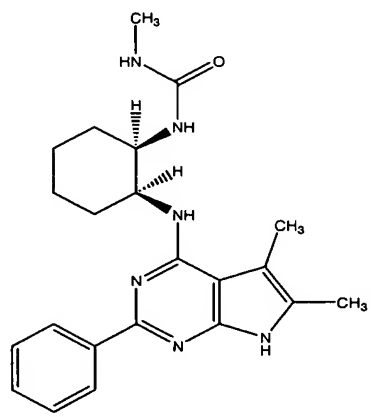
In one embodiment of the compound, the compound has the structure:



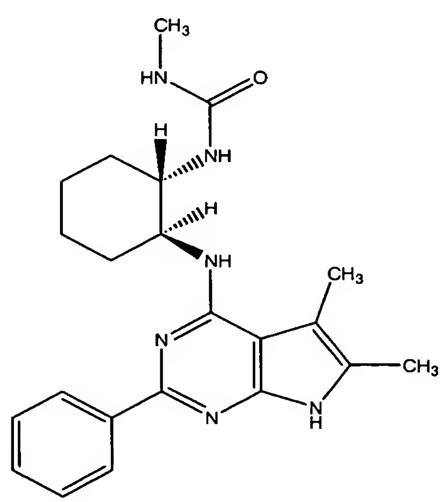
In another embodiment of the compound, the compound has the structure:



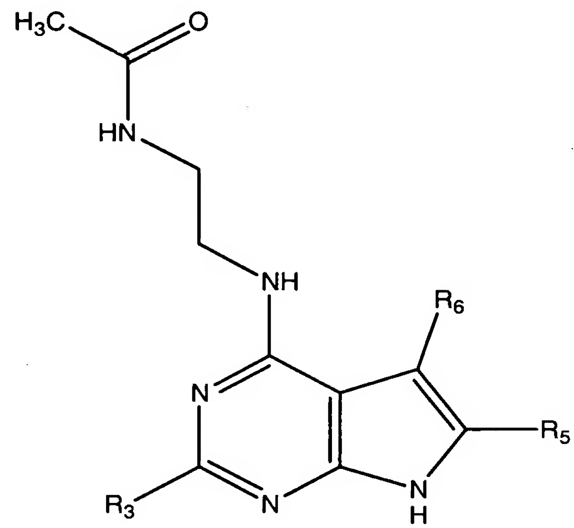
In another embodiment of compound 1500, the compound has the structure:



In a further embodiment of the compound, the compound has the structure:



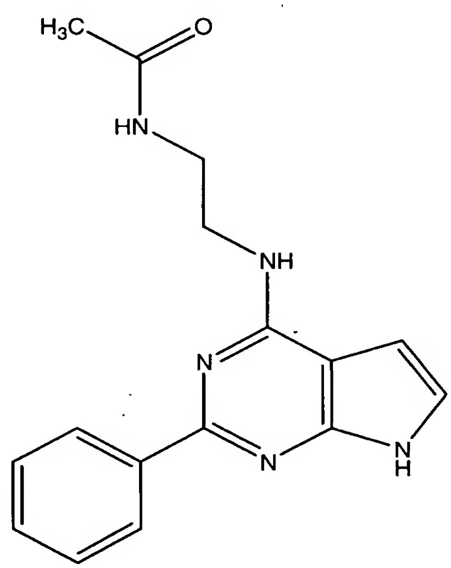
This invention also provides a compound having the structure:



VII

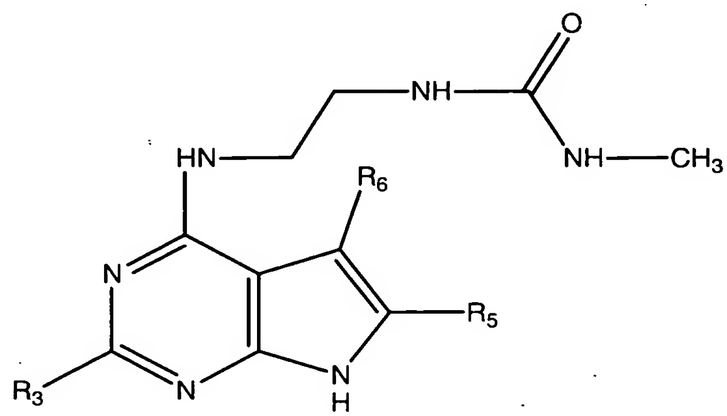
wherein R_3 is a 5-6 membered aromatic ring; wherein R_5 and R_6 are independently H, or alkyl; with the proviso that R_3 is not 4-pyridyl.

In one embodiment of the compound, the compound has the structure:



(Compound 1501)

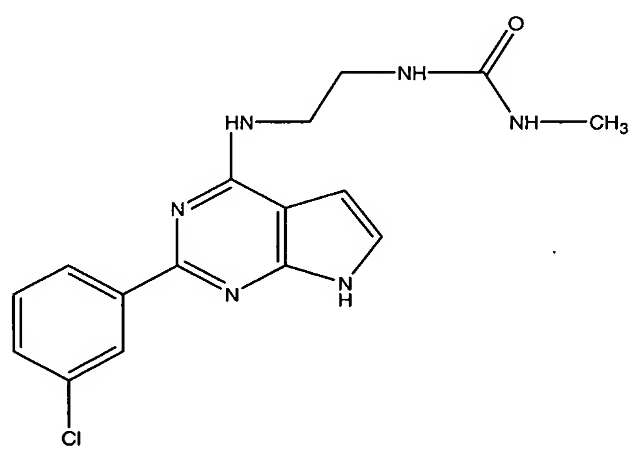
This invention further provides a compound having the structure:



VIII

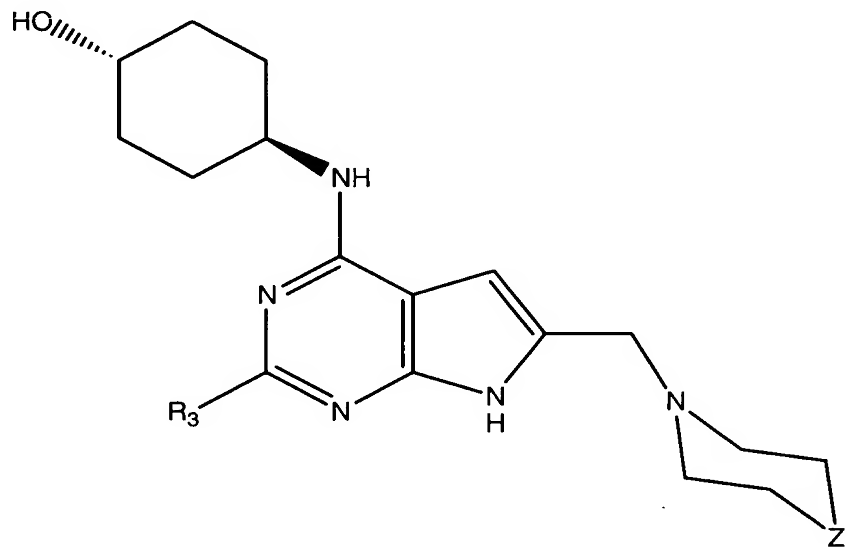
wherein R_3 is a substituted 5-6 membered aromatic ring; wherein R_5 and R_6 are independently H, or alkyl.

In one embodiment of the compound, the compound has the structure:



(Compound 1520)

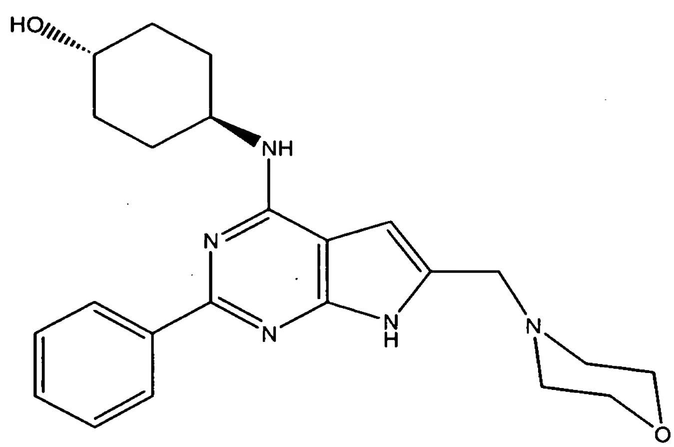
This invention also provides a compound having the structure:



IX

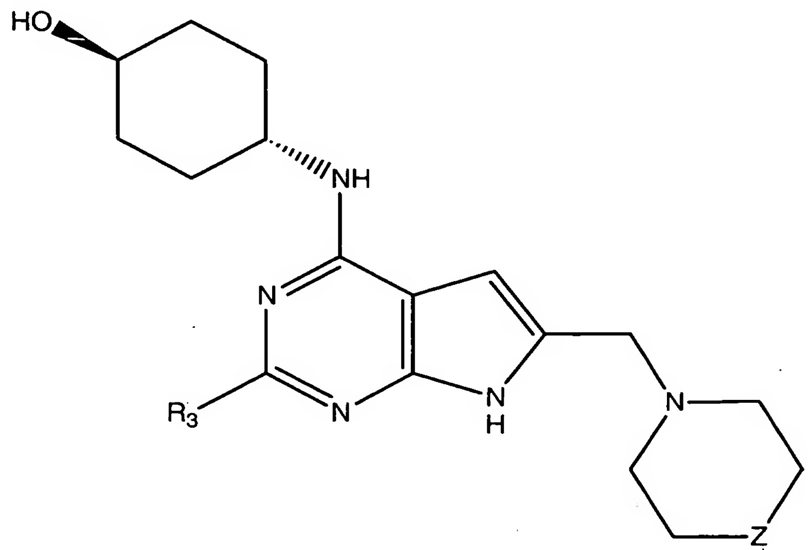
wherein R_3 is a 5-6 membered aromatic ring; wherein Z is oxygen, or sulfur.

In one embodiment of the compound, the compound has the structure:



(Compound 1503)

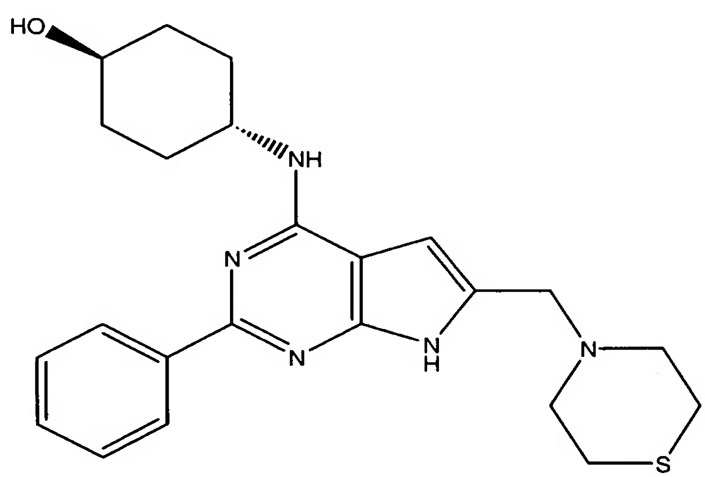
This invention also provides a compound having the structure:



X

wherein R_3 is a 5-6 membered aromatic ring; wherein Z is oxygen, or sulfur.

In one embodiment of the compound, the compound has the structure:



(Compound 1504)

This invention further provides a method for treating a disease associated with A_1 adenosine receptor in a subject, comprising administering to the subject a therapeutically effective amount of a compound having the formula IV, V, VI, VII, VIII, IX, or X.

In one embodiment of the method, the subject is a mammal. In another embodiment of the method, the mammal is a human.

In another embodiment of the method, the A_1 adenosine receptor is associated with cognitive disease, renal failure, cardiac arrhythmias, respiratory epithelia, transmitter release, sedation, vasoconstriction, bradycardia, negative cardiac inotropy and dromotropy, branchoconstriction, neutropil chemotaxis, reflux condition, or ulcerative condition.

This invention also provides a combination therapy for asthma, comprising compounds IV and V, and a steroid, b2 agonist, glucocoticoid, lucotriene antagonist, or anticolinegic agonist. Diseases associated with adenosine A_1, A_{2a}, A_{2b} and A_3 receptors are disclosed in WO 99/06053 and WO-09822465, WO-09705138, WO-09511681, WO-09733879, JP-09291089, PCT/US98/16053 and U.S. Patent No. 5,516,894, the entire content of which are fully incorporate herein by reference.

This invention also provides a water-soluble prodrug of a compound having the structures IV, V, VI, VII, VIII, IX, or X, wherein said water-soluble prodrug that is metabolized *in vivo* to an active drug which selectively inhibit A_1 adenosine receptor.

In one embodiment of the prodrug, said prodrug is metabolized *in vivo* by esterase catalyzed hydrolysis.

This invention also provides a pharmaceutical composition

comprising the prodrug and a pharmaceutically acceptable carrier.

This invention further provides a method for inhibiting the activity of an A_1 adenosine receptor in a cell, which comprises contacting said cell with a compound having the structures IV, V, VI, VII, VIII, IX, or X.

In one embodiment of the method, the compound is an antagonist of said A_1 adenosine receptor.

This invention also provides for a method for treating a gastrointestinal disorder in an subject, comprising administering to the an effective amount of a compound having the structures IV, V, VI, VII, VIII, IX, or X.

In one embodiment of the method, said disorder is diarrhea.

In another embodiment of the method, the subject is a human.

In another embodiment of the method, the compound is an antagonist of A_1 adenosine receptors.

This invention also provides a method for treating respiratory disorder in a subject, comprising administering to the subject an effective amount of a compound having the structures IV, V, VI, VII, VIII, IX, or X.

In one embodiment of the method, said disorder is asthma, chronic obstructive pulmonary disease, allergic rhinitis, or an upper respiratory disorder.

In another embodiment of the method, the subject is a human.

In another embodiment of the method, said compound is an

antagonist of A_1 adenosine receptors.

This invention further provides a method for treating damage to the eye of a subject which comprises administering to said subject an effective amount of a compound having the structures IV, V, VI, VII, VIII, IX, or X.

In one embodiment of the method, said damage comprises retinal or optic nerve head damage.

In another embodiment of the method, said damage is acute or chronic.

In another embodiment of the method, wherein said damage is the result of glaucoma, edema, ischemia, hypoxia or trauma.

In another embodiment of the method, the subject is a human.

In another embodiment of the method, the compound is an antagonist of A_1 adenosine receptors.

This invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a compound having the structures IV, V, VI, VII, VIII, IX, or X, and a pharmaceutically acceptable carrier.

In another embodiment of the pharmaceutical composition, said therapeutically effective amount is effective to treat a respiratory disorder or a gastrointestinal disorder.

In another embodiment of the pharmaceutical composition, said gastrointestinal disorder is diarrhea.

In another embodiment of the pharmaceutical composition, said respiratory disorder is asthma, allergic rhinitis, or chronic

obstructive pulmonary disease.

In another embodiment of the pharmaceutical composition, said pharmaceutical composition is an ophthalmic formulation.

In another embodiment of the pharmaceutical composition, said pharmaceutical composition is an periocular, retrobulbar or intraocular injection formulation.

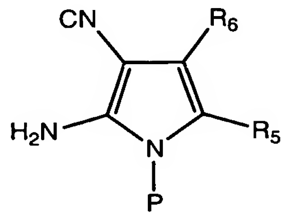
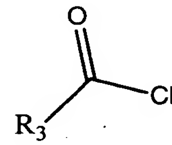
In yet another embodiment of the pharmaceutical composition, said pharmaceutical composition is a systemic formulation.

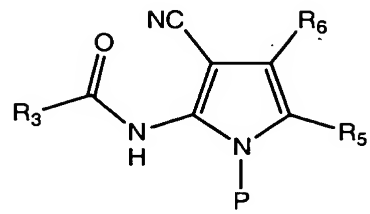
In a further embodiment of the pharmaceutical preparation, said pharmaceutical composition is a surgical irrigating solution.

This invention also provides a packaged pharmaceutical composition for treating a disease associated with A_1 adenosine receptor in a subject, comprising: (a) a container holding a therapeutically effective amount of an adenosine A_1 specific compound; and (b) instructions for using said compound for treating said disease in a subject.

As used herein, "A compound is A_1 selective" means that a compound has a binding constant to adenosine A_1 receptor of at least ten times higher than that to adenosine A_{2a}, A_{2b} or A_3.

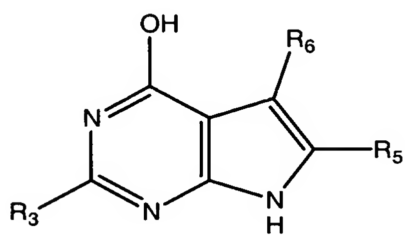
This invention also provides a method of preparing the compound having structure IV, comprising the steps of

a) reacting  and 

to provide 

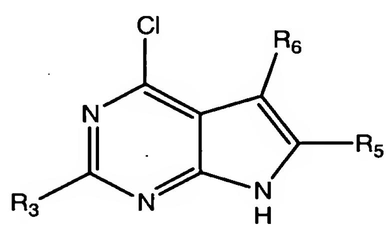
wherein P is a removable protecting group;

b) treating the product of step a) under cyclization conditions to provide



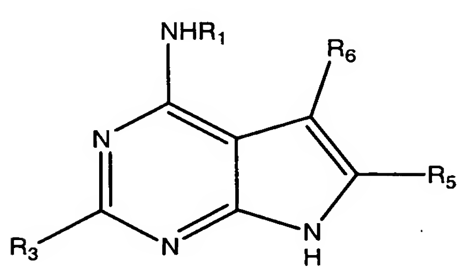
;

c) treating the product of step b) under suitable conditions to provide



; and

d) treating the chlorinated product of step c) with NH_2R_1 to provide

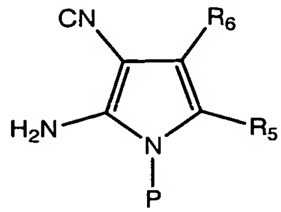
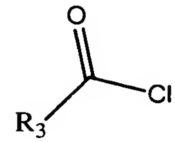


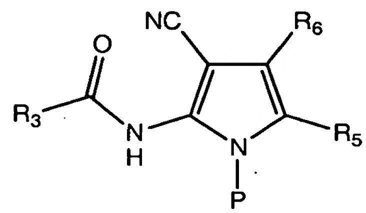
wherein R_1 is *trans*-4-hydroxy cyclohexyl, 2-methylamino carbonylamino cyclohexyl, acetylamino ethyl, or methylamino carbonylamino ethyl;

wherein R_3 is a substituted or unsubstituted four to six membered ring;

wherein R_6 is H, alkyl, substituted alkyl, cycloalkyl; or a pharmaceutically acceptable salt, or a prodrug derivative, or a biologically active metabolite; with the proviso that when R_1 is acetylamino ethyl, R_3 is not 4-pyridyl.

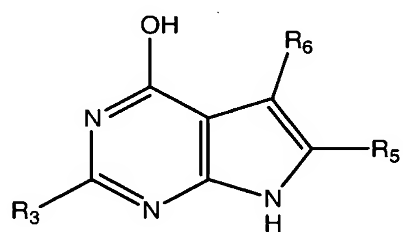
This invention also provides a method of preparing the compound having structure V, comprising the steps of

a) reacting  and 

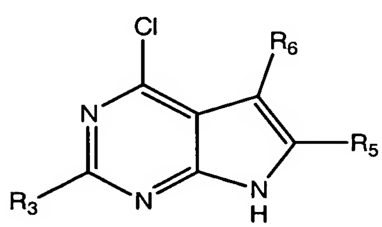
to provide 

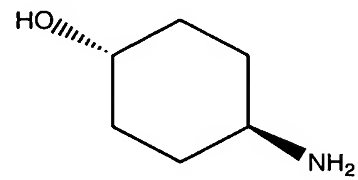
wherein P is a removable protecting group;

b) treating the product of step a) under cyclization conditions to provide

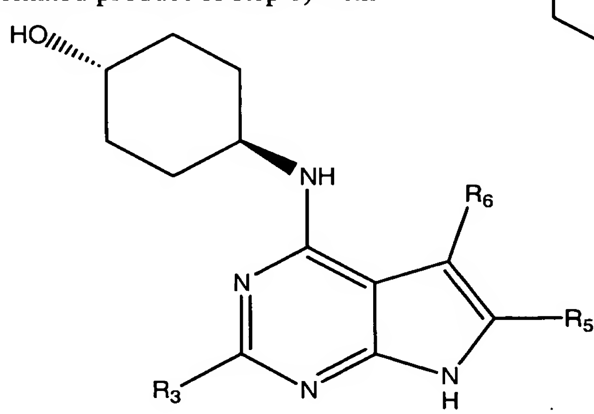
 ;

c) treating the product of step b) under suitable conditions to provide

 ; and

d) treating the chlorinated product of step c) with 

to provide



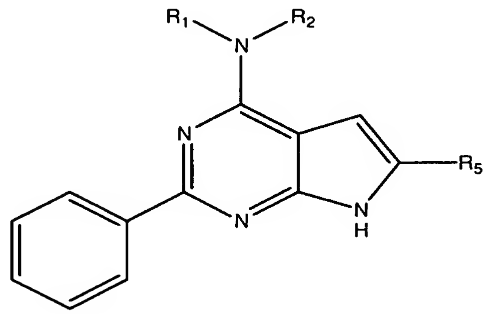
wherein R_3 is aryl, substituted aryl, heteroaryl;

wherein R_5 is H, alkyl, substituted alkyl, aryl, arylalkyl, amino, substituted aryl, wherein said substituted alkyl is $-C(R_7)(R_8)NR_9R_{10}$, wherein R_7 and R_8 are each H or alkyl, wherein R_9 and R_{10} are each alkyl or cycloalkyl, or NR_9R_{10} is a ring system of between 4 and 7 members; and

wherein R_6 is H, alkyl, substituted alkyl, or cycloalkyl.

Compounds represented by formulas VI, VII, and VIII can be synthesized by any of the Schemes I-VIII. Compounds represented by formulas IX and X can be prepared by Scheme IX.

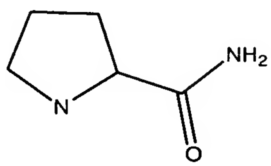
This invention further provides compounds having the formula:



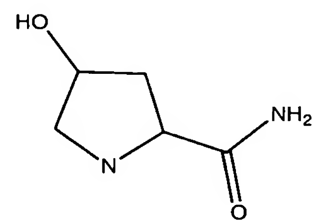
XI

wherein

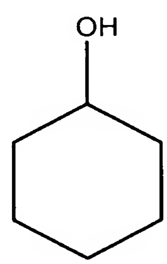
R_1NR_2 together form a ring having the structure:



or



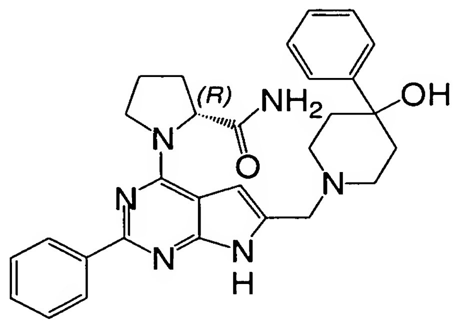
or R_1 is H and R_2 is:



;

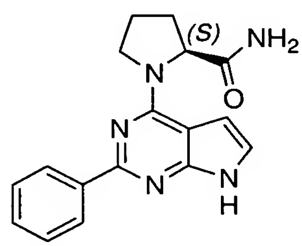
R_5 is H, or substituted or unsubstituted alkyl or alkylaryl.

In one embodiment the compound has the structure:



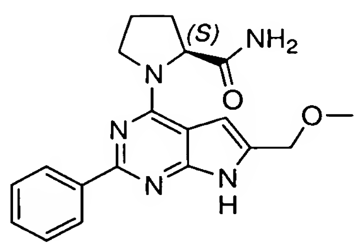
1601

In a further embodiment the compound has the structure:



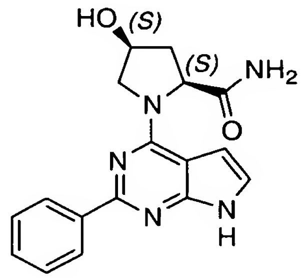
1602

In a further embodiment the compound has the structure:



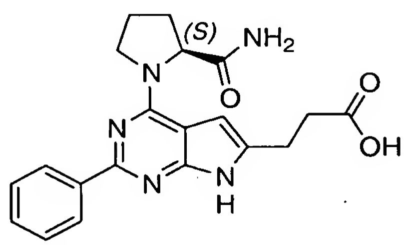
1605

In a further embodiment the compound has the structure:



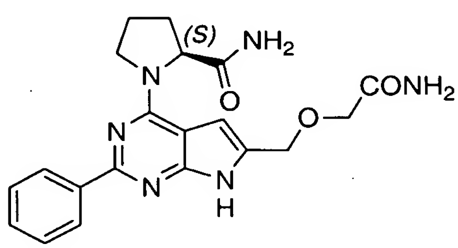
1606

In a further embodiment the compound has the structure:



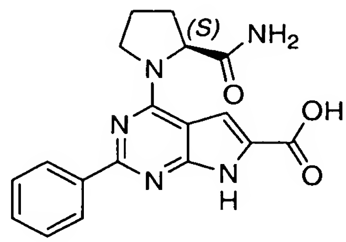
1611

In a further embodiment the compound has the structure:



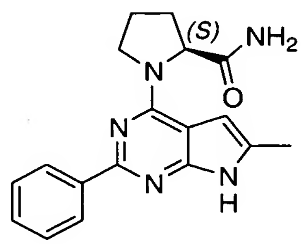
1614

In a further embodiment the compound has the structure:



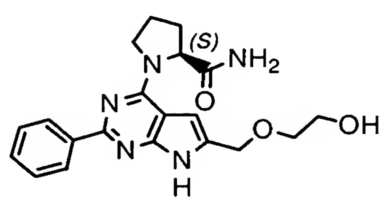
1619

In a further embodiment the compound has the structure:



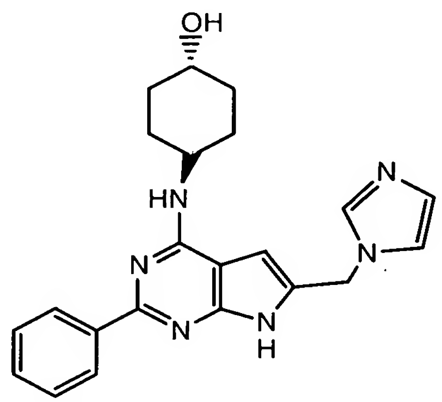
1621

In a further embodiment the compound has the structure:



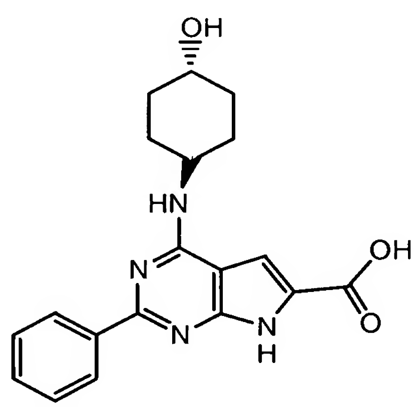
1623

In a further embodiment the compound has the structure:



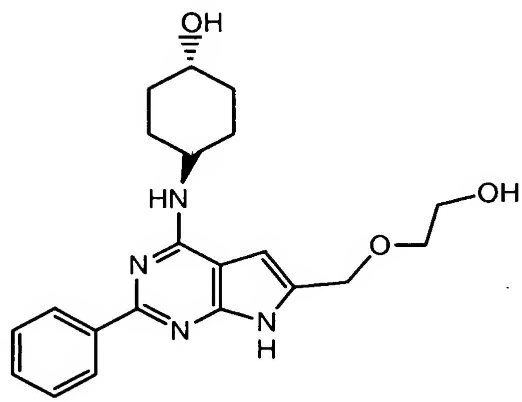
1624

In a further embodiment the compound has the structure:



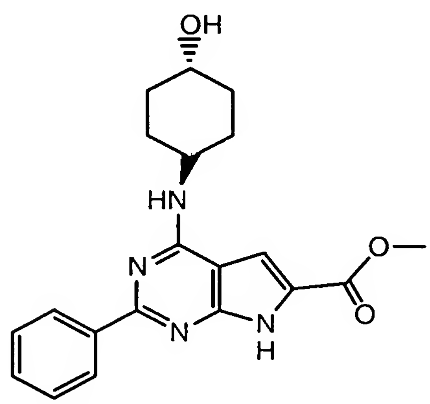
1625

In a further embodiment the compound has the structure:



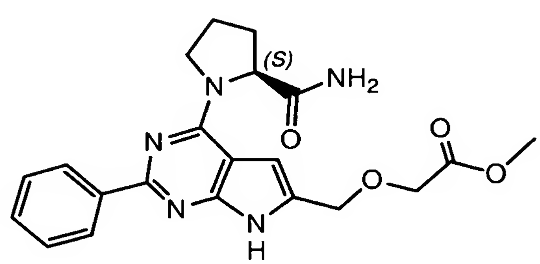
1626

In a further embodiment the compound has the structure:



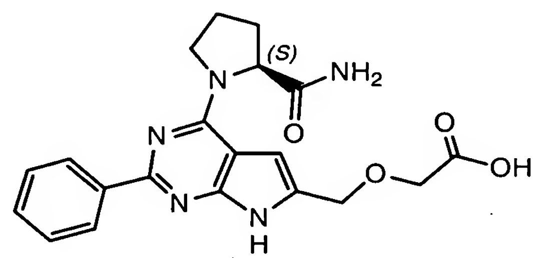
1627

In a further embodiment the compound has the structure:



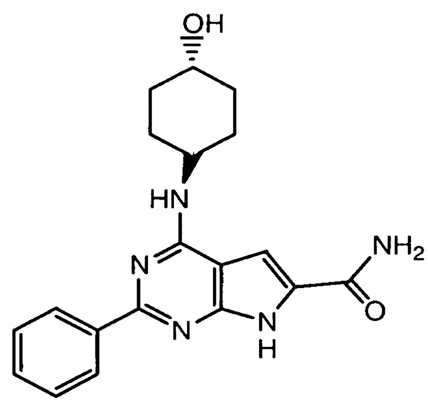
1628

In a further embodiment the compound has the structure:



1629

In a further embodiment the compound has the structure:



1630

In a further embodiment the invention provides a method for treating a disease associated with A_1 adenosine receptor in a subject, comprising administering to the subject a therapeutically effective amount of a compound of formula XI, or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630.

In a further embodiment the invention provides the above method, wherein the subject is a mammal.

In a further embodiment the invention provides the above method, wherein the mammal is a human.

In a further embodiment the invention provides the above method, wherein said A_1 adenosine receptor is associated with cognitive disease, renal failure, cardiac arrhythmias, respiratory epithelia, transmitter release, sedation, vasoconstriction, bradycardia, negative cardiac inotropy and dromotropy, branchoconstriction, neutropil chemotaxis, reflux condition, or ulcerative condition.

In a further embodiment the invention provides a water-soluble prodrug of the compounds of formula XI, or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630, wherein the water-soluble prodrug is metabolized in vivo to produce an active drug which selectively inhibits A_1 adenosine receptor.

In a further embodiment the invention provides, wherein said prodrug is metabolized in vivo by esterase catalyzed hydrolysis.

In a further embodiment the invention provides a pharmaceutical composition comprising the above prodrug and a pharmaceutically acceptable carrier.

In a further embodiment the invention provides a method for inhibiting the activity of an A_1 adenosine receptor in a cell, which comprises contacting the cell with a compound of formula XI or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630.

In a further embodiment the invention provides the above method for inhibiting the activity of an A_1 adenosine receptor in a cell, wherein the compound is an antagonist of the A_1 adenosine receptor.

In a further embodiment the invention provides the above method for inhibiting the activity of an A_1 adenosine receptor in a cell, wherein the cell is human cell.

In a further embodiment the invention provides the above method for inhibiting the activity of an A_1 adenosine receptor in a human cell, wherein the compound is an antagonist of A_1 adenosine receptors.

In a further embodiment the invention provides a method for treating a disease associated with A_1 adenosine receptor in a subject, wherein said disease is asthma, chronic obstructive pulmonary disease, allergic rhinitis, or an upper respiratory disorder.

In a further embodiment the invention provides a method for treating a disease associated with A_1 adenosine receptor in a subject, wherein said disease is asthma, chronic obstructive pulmonary disease, allergic rhinitis, or an upper respiratory disorder and wherein the subject is a human.

In a further embodiment the invention provides a method for treating the above disease, wherein said compound is an antagonist of A1 adenosine receptors.

In a further embodiment the invention provides a combination therapy for asthma, comprising a compound of formula XI, or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630, and a steroid, b2 agonist, glucocorticoid, lucotriene antagonist, or

anticolinergic agonist.

In a further embodiment the invention provides a pharmaceutical composition comprising a therapeutically effective amount of a compound of formula XI, or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630, and a pharmaceutically acceptable carrier.

In a further embodiment the invention provides a method for treating a respiratory disorder a compound of formula XI, or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630, wherein said respiratory disorder is asthma, allergic rhinitis, or chronic obstructive pulmonary disease.

In a further embodiment the invention provides the above pharmaceutical composition(s), wherein said pharmaceutical composition is an periocular, retrobulbar or intraocular injection formulation.

In a further embodiment the invention provides the above pharmaceutical composition(s), wherein said pharmaceutical composition is a systemic formulation.

In a further embodiment the invention provides the above pharmaceutical composition(s), wherein said pharmaceutical composition is a surgical irrigating solution.

In a further embodiment the invention provides a packaged pharmaceutical composition for treating a disease associated with A1 adenosine receptor in a subject, comprising:

(a) a container holding a therapeutically effective amount of a compound of formula XI, or compound

1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630; and

(b) instructions for using said compound for treating said disease in a subject.

In a further embodiment the invention provides a pharmaceutically acceptable salt a compound of formula XI, or compound 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, or 1630.

In a further embodiment the invention provides the above pharmaceutically acceptable salt, wherein the pharmaceutically acceptable salt of compound 1611, 1619, 1625, 1628, or 1629 contains a cation selected from the group consisting of sodium, calcium and ammonium.

In yet a further embodiment the invention provides a method for treating a disease associated with A_1 adenosine receptor in a subject, wherein the A_1 adenosine receptor is associated with congestive heart failure.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application, including those referenced in the background section, are hereby incorporated by reference. It should be understood that the models used throughout the examples are accepted models and that the demonstration of efficacy in these models is predictive of efficacy in humans.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will

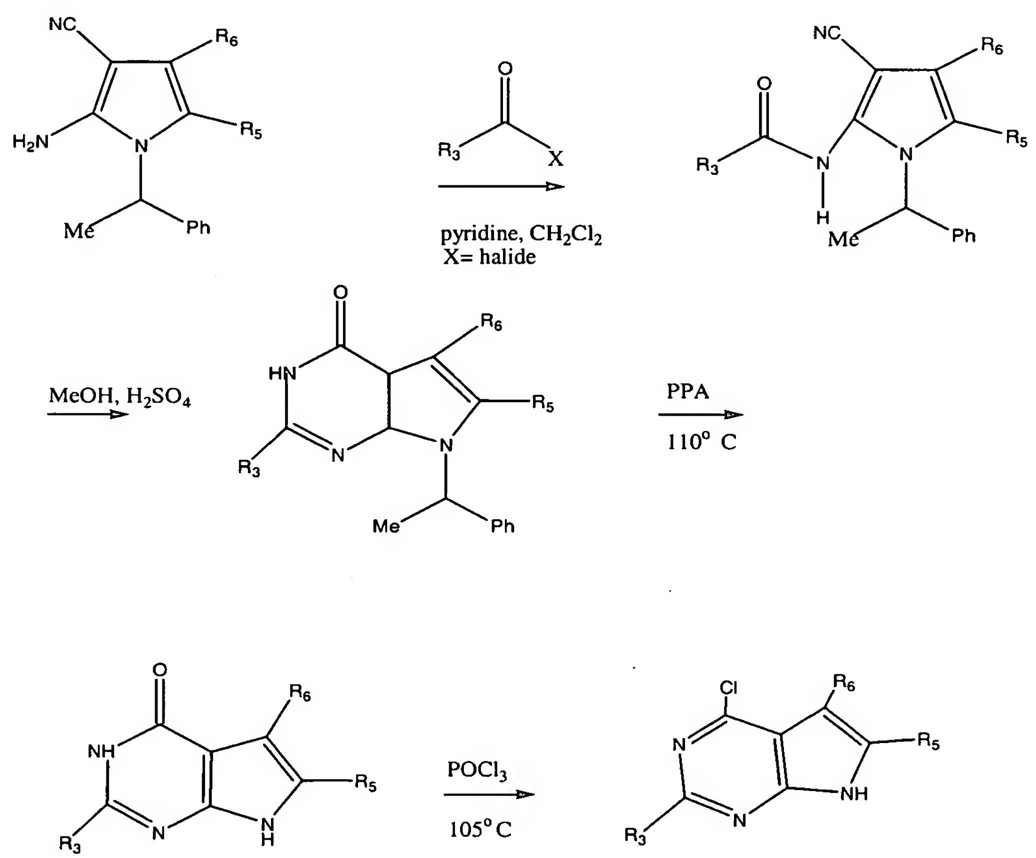
readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

The deazapurines of the invention can be prepared using standard methods for organic synthesis. Deazapurines can be purified by reverse phase HPLC, chromatography, recrystallization, etc. and their structures confirmed by mass spectral analysis, elemental analysis, IR and/or NMR spectroscopy.

Typically, synthesis of the intermediates as well as the deazapurines of the invention is performed in solution. The addition and removal of one or more protecting group is also typical practice and is known to those skilled in the art. Typical synthetic schemes for the preparation of deazapurine intermediates of the invention are outlined below in Scheme I.

Scheme I

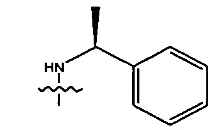
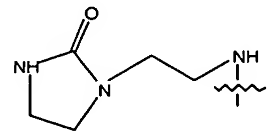
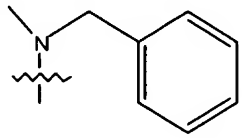
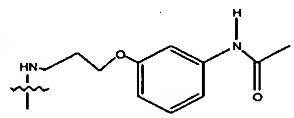
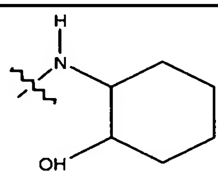
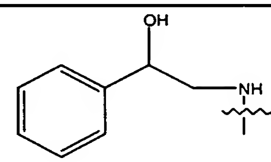
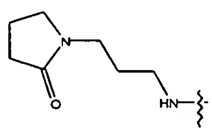
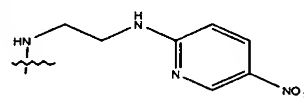
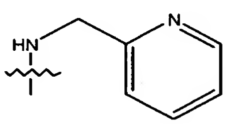
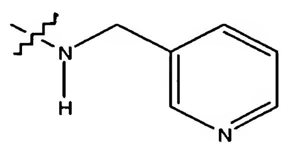
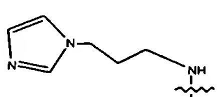
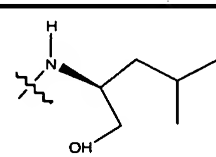
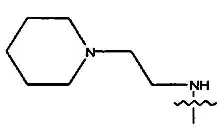
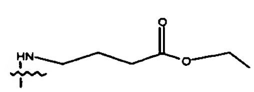


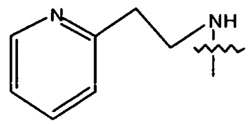
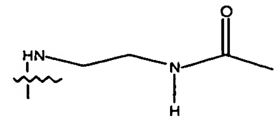
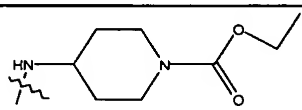
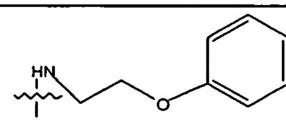
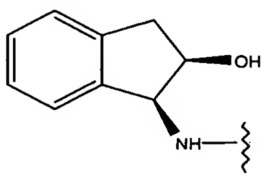
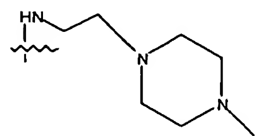
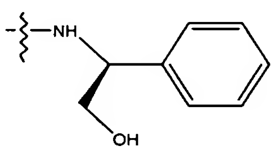
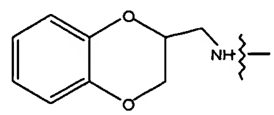
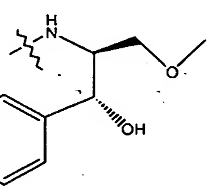
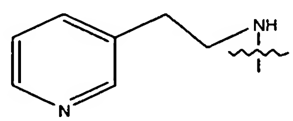
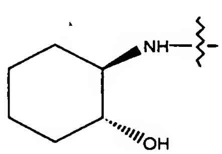
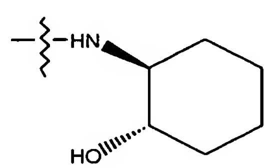
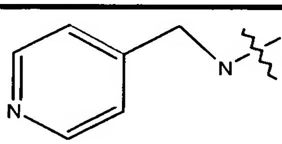
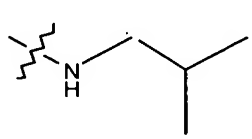
wherein R_3, R_5 and R_6 are as defined above.

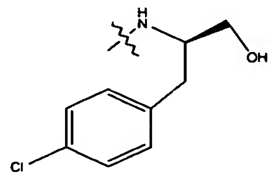
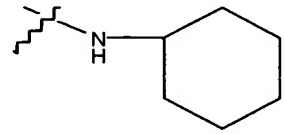
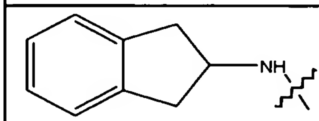
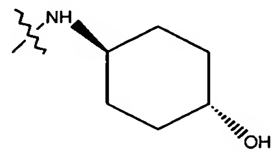
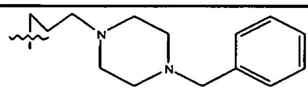
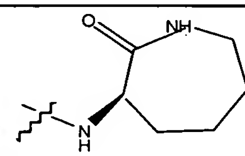
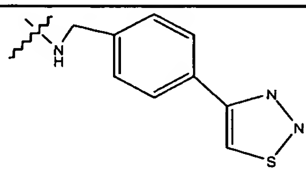
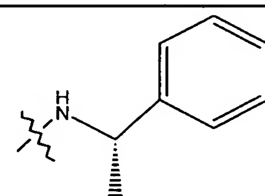
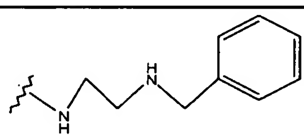
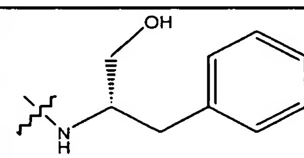
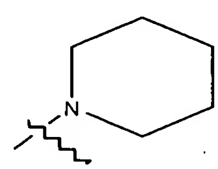
In general, a protected 2-amino-3-cyano-pyrrole can be treated with an acyl halide to form a carboxyamido-3-cyano-pyrrole which can be treated with acidic methanol to effect ring closure to a pyrrolo[2,3d]pyrimidine-4(3*H*)-one (Muller, C.E. *et al.* J. Med. Chem. 40:4396 (1997)). Removal of the pyrrolo protecting group followed by treatment with a chlorinating reagent, *e.g.*, phosphorous oxychloride, produced substituted or unsubstituted 4-chloro-7H-pyrrolo[2,3d]pyrimidines. Treatment of the chloropyrimidine with amines afforded 7-deazapurines.

For example, as shown in Scheme I, a N-(1-*dl*-phenylethyl)-2-amino-3-cyano-pyrrole was treated with an acyl halide in pyridine and dichloromethane. The resultant N-(1-*dl*-phenylethyl)-2-phenylcarboxyamido-3-cyano-pyrrole was treated with a 10:1 mixture of methanol/sulfuric acid to effect ring closure, resulting in a *dl*-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine-4(3*H*)-one. Removal of the phenylethyl group by treatment of the pyrimidine with polyphosphoric acid (PPA) followed by $POCl_3$ afforded a key intermediate, the 4-chloro-7H-pyrrolo[2,3d]pyrimidine. Further treatment of the 4-chloro-7H-pyrrolo[2,3d]pyrimidine with various amines listed in Table 1-A gives compounds of formula (I) and (II).

TABLE 1-A

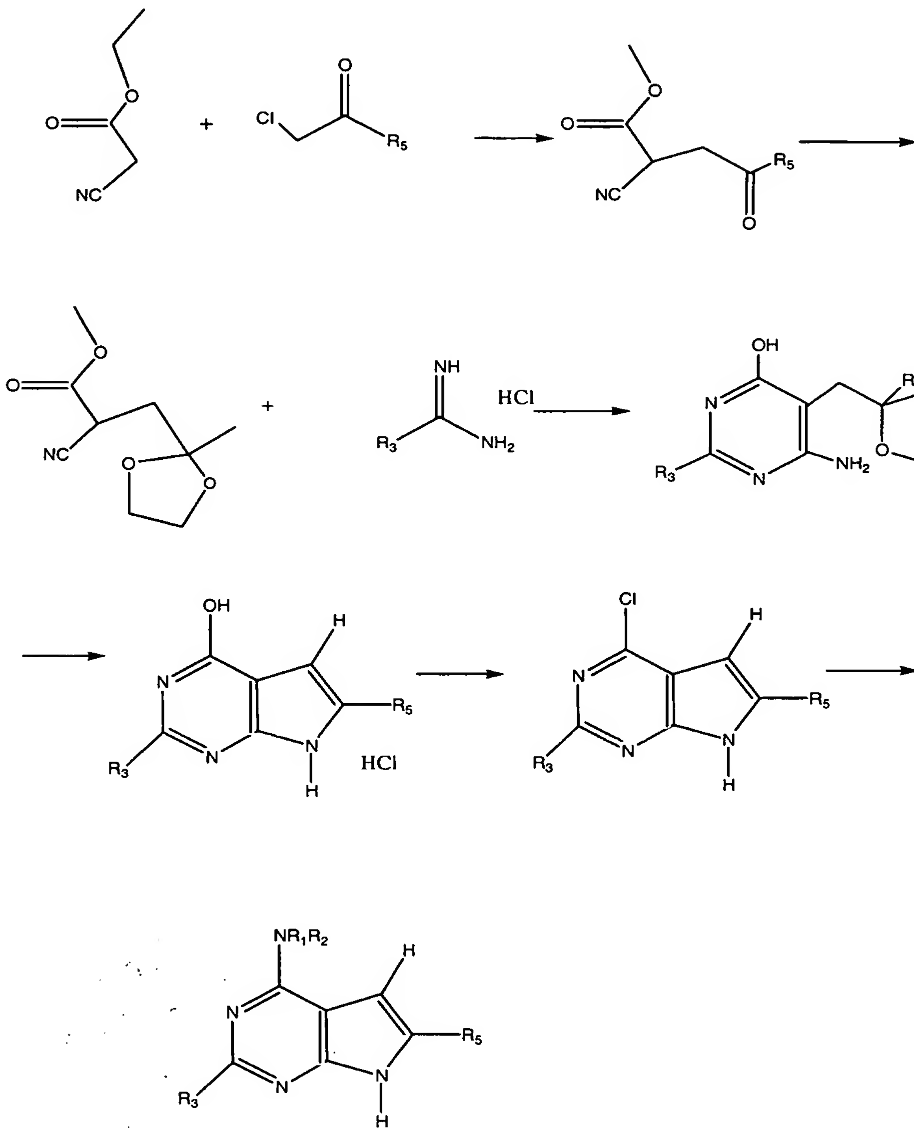
R	$M^+ + H$	R	$M^+ + H$
	343.2		351.27
	343.18		430.35
	337.21		359.44
	364.19		404.32
	330.18		330.45
	347.22		339.47
	350.28		353.41

	344.19		324.45
	394.16		359.38
	371.12		379.40
	359.39		387.41
	403.33		344.48
	351.49		337.53
	330.37		295.2

	407.23		321.2
	355.45		337.53
	441.33		350.2
	413.24		343.2
	372.48		373.2
			307.2

A general approach to prepare 6-substituted pyrroles is depicted in the following scheme (Scheme II).

Scheme II



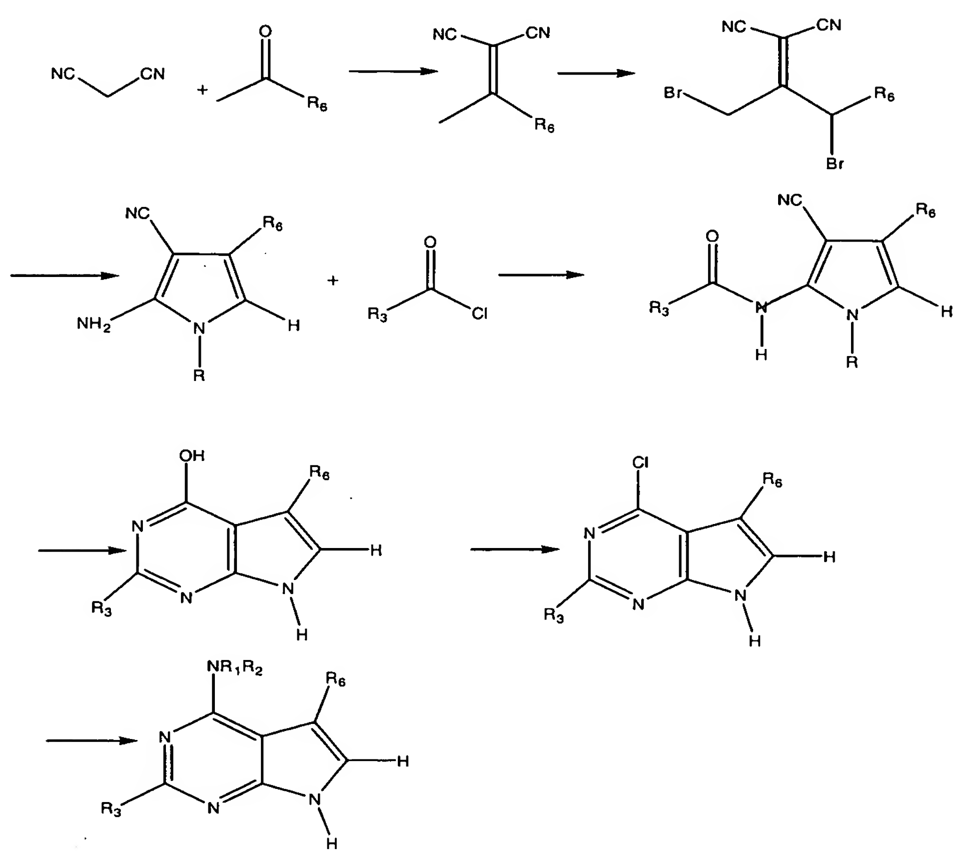
wherein R_1 through R_5 are as defined above.

Transesterification and alkylation of ethyl cyanoacetate with an α-haloketone affords a ketomethylester. Protection of the ketone followed by treatment with an amidine (*e.g.*, alkyl, aryl or alkylaryl) hydrochloride produced the resultant ketal protected pyrimidine. Removal of the protecting group, followed by cyclization and treatment with phosphorous

oxychloride afforded the chloride intermediate which could be further treated with an amine to afford an amine 6-substituted pyrrole. Additionally, alkylation of the pyrrole nitrogen can be achieved under art recognized conditions.

A general approach to prepare 5-substituted pyrroles is depicted in the following scheme (Scheme III).

Scheme III

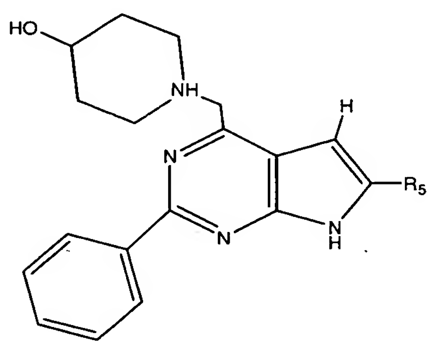


wherein R_1 through R_6 are defined as above and R is a removable protecting group.

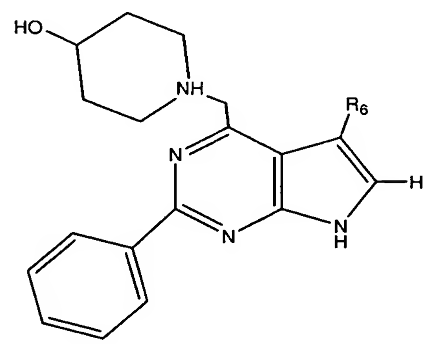
Condensation of malononitrile and an excess of a ketone followed by bromination of the product afforded a mixture of starting material, monobrominated and dibrominated products which were treated with an alkylamine, arylamine or

alkylarylamine. The resultant amine product was acylated with an acid chloride and the monacylated pyrrole was cyclized in the presence of acid to afford the corresponding pyrimidine. The pyrrole protecting group was removed with polyphosphoric acid and treated with phosphorous oxychloride to produce a chlorinated product. The chlorinated pyrrole could subsequently be treated with an amine to produce an amino 5-substituted pyrrole. Alkylation of the pyrrole nitrogen can be achieved under art recognized conditions.

Schemes IV and V depict methods for preparing the deazapurines 1 and 2 of the invention.



1



2

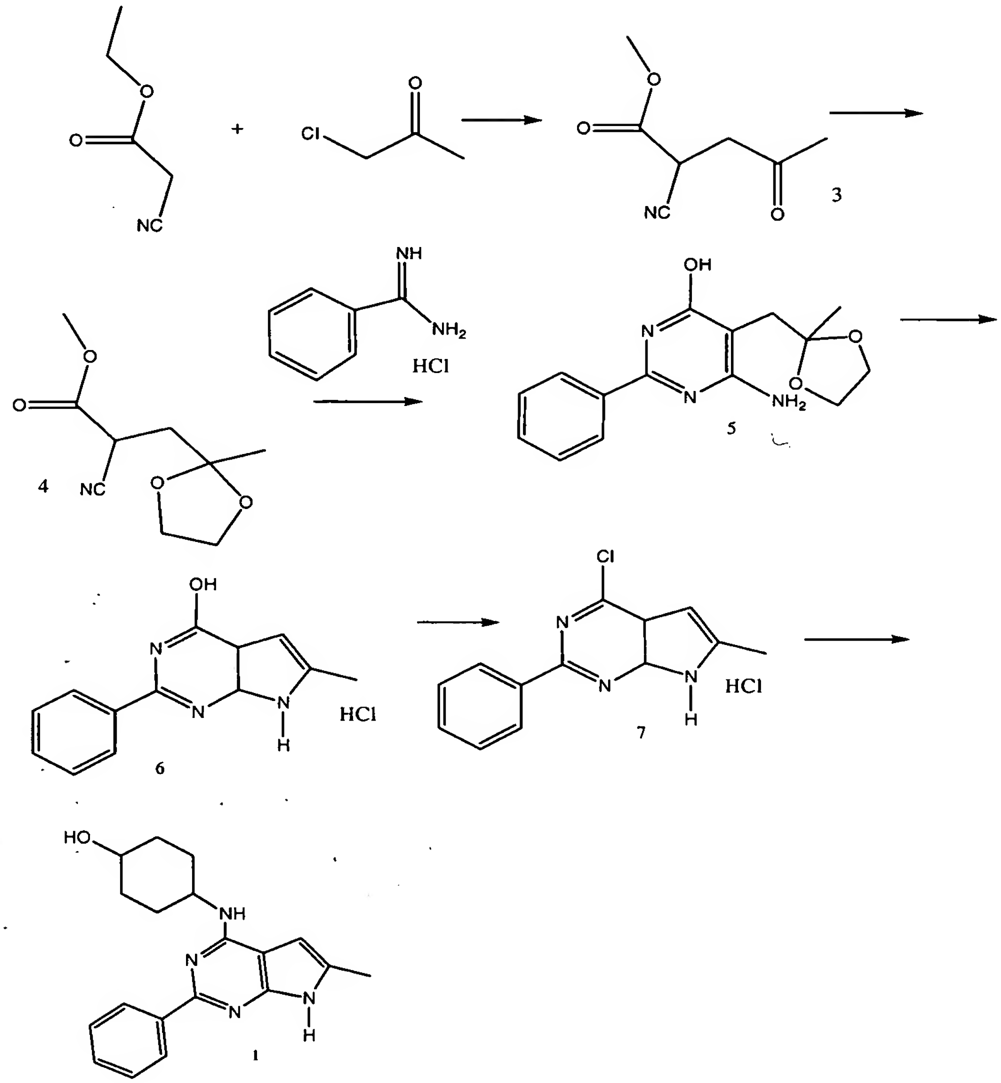
wherein R_5 and R_6 are as described above, *e.g.*, CH_3.

Specific Preparation of 6-methyl pyrrolopyrimidines:

The key reaction toward 6-methylpyrrolopyrimidines (1) [R_5 = CH_3] was cyclization of a cyanoacetate with benzamidine to a pyrimidine. It was believed methyl cyanoacetate would cyclize more efficiently with benzamidine to a pyrimidine than the corresponding ethyl ester. Therefore, transesterification and alkylation of ethyl cyanoacetate in the presence of NaOMe and an excess of an α-haloacetyl moiety, *e.g.*, chloroacetone, gave

the desired methyl ester (3) in 79% yield (Scheme IV). The ketoester (3) was protected as the acetal (4) in 81% yield. A new cyclization method to the pyrimidine (5) was achieved with an amidine hydrochloride, *e.g.*, benzamidine hydrochloride, with 2 equivalents of DBU to afford the 5 in 54% isolated yield. This method improves the yield from 20% using the published conditions, which utilizes NaOMe during the cyclization with guanidine. Cyclization to the pyrrole-pyrimidine (6) was achieved via deprotection of the acetal in aqueous HCl in 78% yield. Reaction of (6) with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative (7). Coupling with *trans*-4-aminocyclohexanol in dimethyl sulfoxide at 135°C gave (1) in 57% from (7). One skilled in the art will appreciate that choice of reagents allows for great flexibility in choosing the desired substituent R_5.

Scheme IV


O
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HCl
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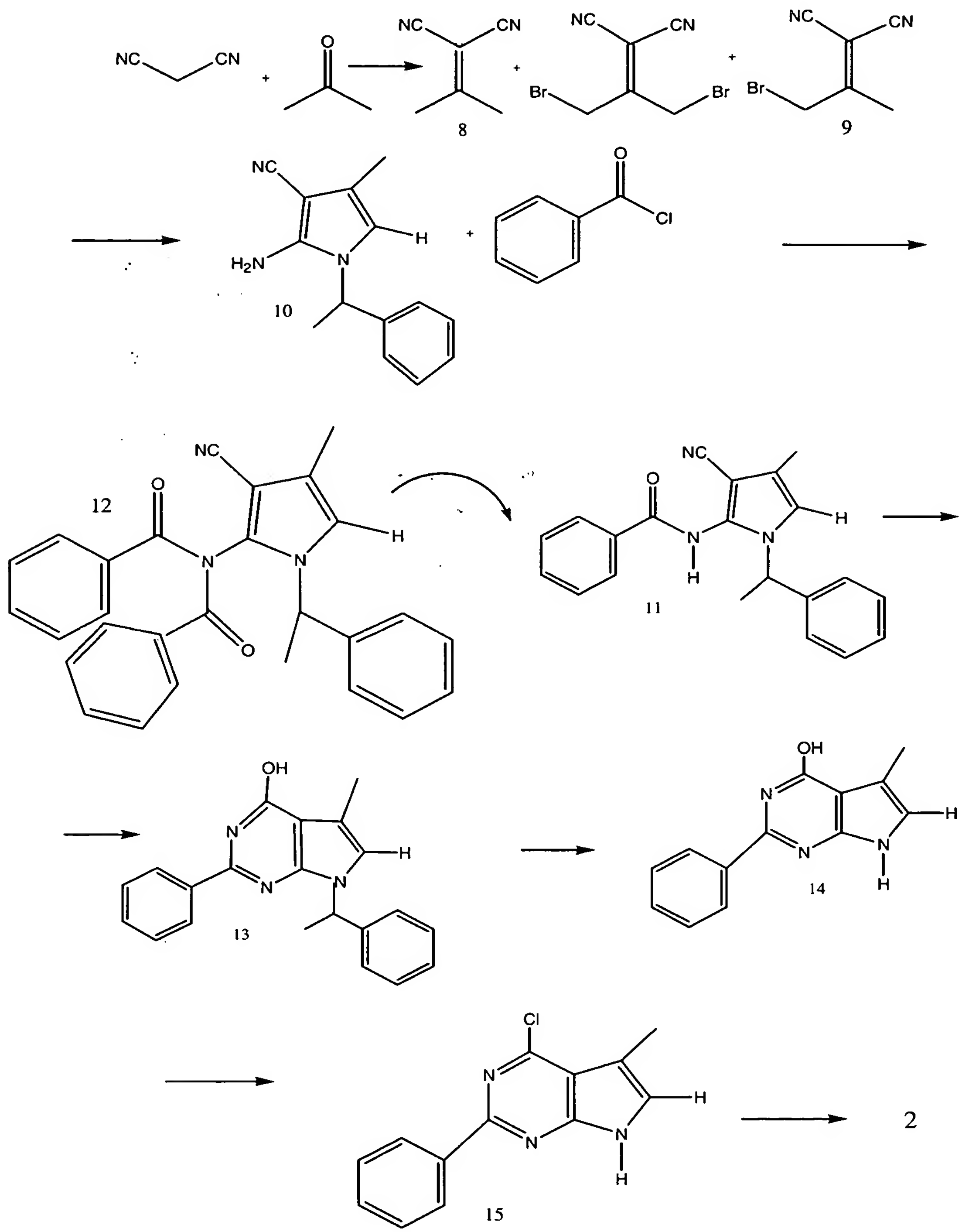
1

Specific Preparation of 5-methylpyrrolopyrimidines

Knoevengel condensation of malononitrile and an excess ketone, *e.g.*, acetone in refluxing benzene gave 8 in 50% yield after distillation. Bromination of 8 with *N*-bromosuccinimde in the presence of benzoyl peroxide in chloroform yielded a mixture of starting material, mono- (9), and di-brominated products (5/90/5) after distillation (70%). The mixture was reacted with an α-methylalkylamine or α-methylarylamine, *e.g.*, α-methylbenzylamine, to deliver the aminopyrrole (10). After passing through a short silica gel column, the partially purified amine (31% yield) was acylated with an acid chloride, *e.g.*, benzoyl chloride to deliver mono- (11), and diacylated (12) pyrroles, which were separated by flash chromatography. Acid hydrolysis of the disubstituted pyrrole (12) generated a combined yield of 29% for the acylpyrrole (11). Cyclization in the presence of concentrated sulphuric acid and DMF yielded (13) (23%), which was deprotected with polyphosphoric acid to (14). Reaction of (14) with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative (15). Coupling with *trans*-4-aminocyclohexanol in dimethyl sulfoxide at 135°C gave (2) [R_6 = CH_3] in 30% from (14) (See Scheme V). One skilled in the art will appreciate that choice of reagents allows for great flexibility in choosing the desired substituent R_6.

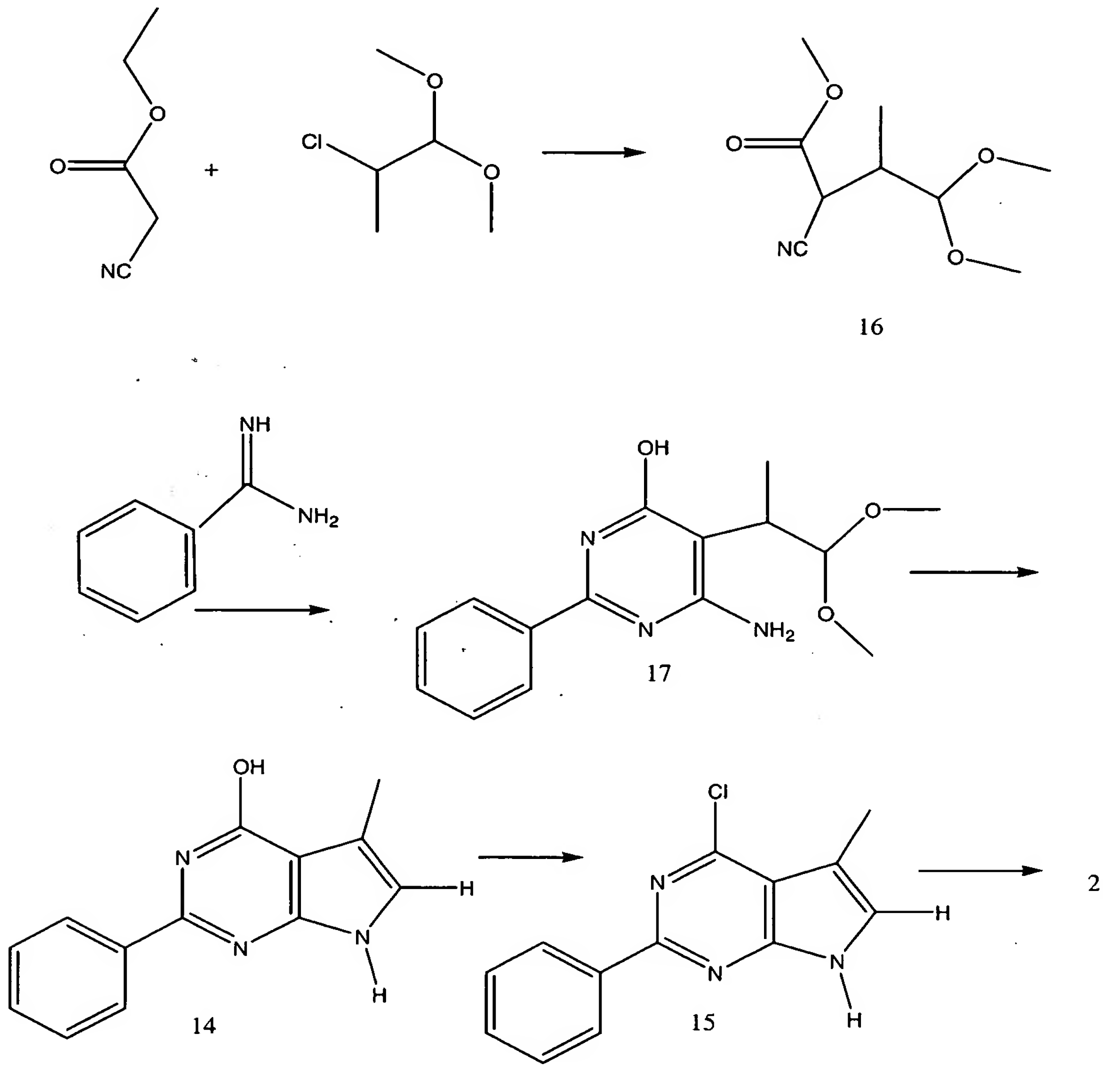
Scheme V



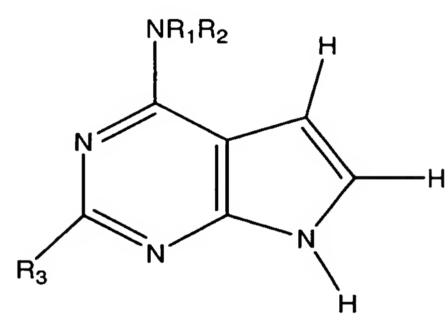
Alternative Synthetic Route to R_6-Substituted Pyrroles, *e.g.*, 5-methyl pyrrolopyrimidines:

This alternative route to R_6-substituted pyrroles, *e.g.*, 5-methylpyrrolopyrimidines, involves transesterification and alkylation of ethyl cyanoacetate to (16) (Scheme VI). The condensation of (16) with benzamidine hydrochloride with 2 equivalents of DBU affords the pyrimidine (17). Cyclization to the pyrrole-pyrimidine (14) will be achieved via deprotection of the acetal in aqueous HCl. Reaction of (14) with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative (15). Coupling with *trans*-4-aminocyclohexanol in dimethyl sulfoxide at 135°C gives 2. This procedure reduces the number of synthetic reactions to the target compound (2) from 9 to 4 steps. Moreover, the yield is dramatically improved. Again, one skilled in the art will appreciate that choice of reagents allows for great flexibility in choosing the desired substituent R_6.

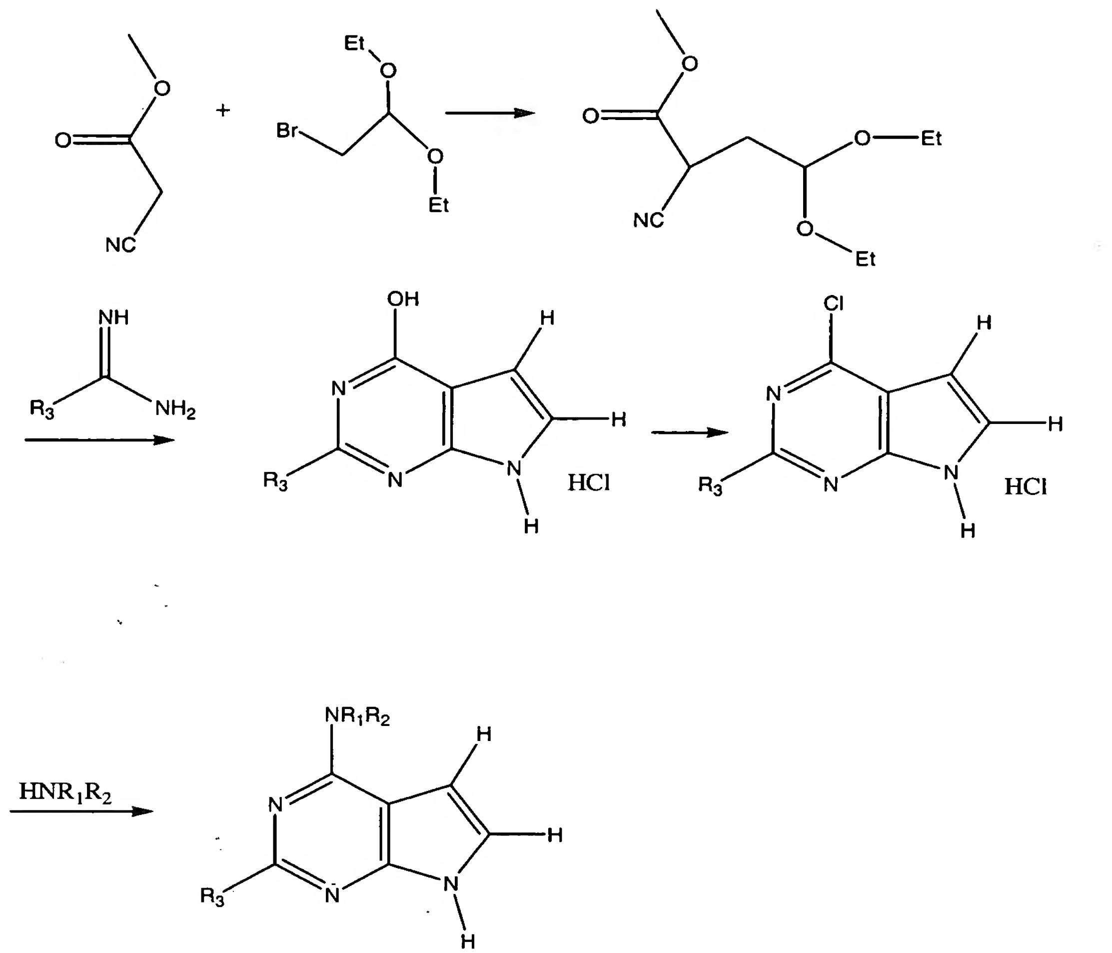
Scheme VI



A general approach to prepare *des*-methyl pyrrole is depicted in the following scheme (Scheme VII)



Scheme VII

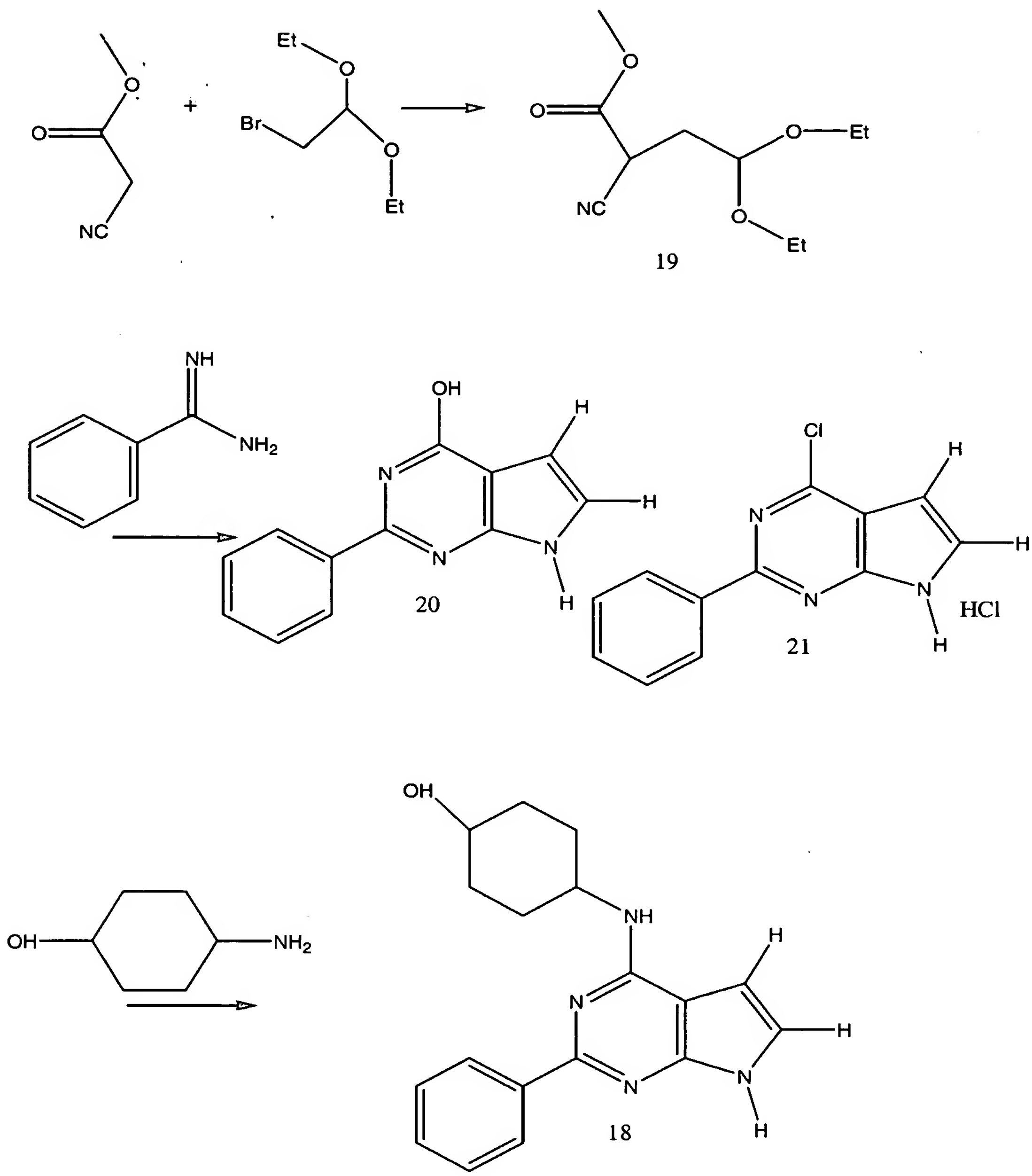


wherein R_1 through R_3 are defined as above.

Alkylation of an alkyl cyanoacetate with a diethyl acetal in the presence of a base afforded a cyano diethyl acetal which was treated with an amidine salt to produce a methyl pyrrolopyrimidine precursor. The precursor was chlorinated and treated with an amine to form the *des*-methyl pyrrolopyrimidine target as shown above.

For example, Scheme VIII depicts the synthesis of compound (18).

Scheme VIII

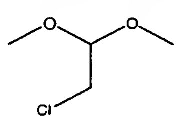
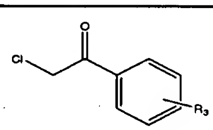
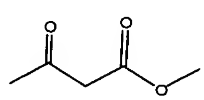
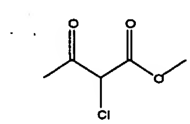
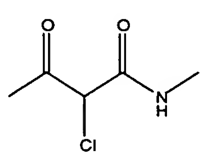


Commercially available methyl cyanoacetate was alkylated with bromoacetaldehyde diethyl acetal in the presence of potassium carbonate and NaI to yield (19). Cyclization to the pyrimidine (20) was achieved in two steps. Initially, the pyrimidine-acetal was formed via reaction of (19) with benzamidine hydrochloride with 2 equivalents of DBU. The resultant pyrimidine-acetal was deprotected without

purification with aqueous 1 N HCl and the resultant aldehyde cyclized to the pyrrolo-pyrimidine (20), which was isolated by filtration. Reaction of (20) with phosphorous oxychloride at reflux afforded the corresponding 4-chloro derivative (21). Coupling of the chloro derivative with *trans*-4-aminocyclohexanol in DMSO at 135°C gave compound (18) from compound (21).

Schemes II-VIII demonstrate that it is possible to functionalize the 5- and 6-position of the pyrrolopyrimidine ring. Through the use of different starting reagents and slight modifications of the above reaction schemes, various functional groups can be introduced at the 5- and 6-positions in formula (I) and (II). Table 2-A illustrates some examples.

Table 2-A. Selected list of 5- and 6-substituted pyrrolopyrimidines.

Starting Reagent	R_5	R_6
	H	
	H	Substituted Ar
	H	$CH_2C(O)OCH_3$
	$C(O)OCH_3$	CH_3
	$C(O)NHCH_3$	CH_3

A skilled artisan will know that metabolism of the compounds disclosed herein in a subject produces certain biologically active metabolites which can serve as drugs.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application, including those referenced in the background section, are hereby incorporated by reference. It should be understood that the models used throughout the examples are accepted models and that the demonstration of efficacy in these models is predictive of efficacy in humans.

Exemplification

Preparation 1:

A modification of the alkylation method of Seela and Lüpke was used.[1] To an ice-cooled (0°C) solution of ethyl cyanoacetate (6.58 g, 58.1 mmol) in MeOH (20 mL) was slowly added a solution of NaOMe (25% w/v; 58.1 mmol). After 10 min, chloroacetone (5 mL; 62.8 mmol) was slowly added. After 4 h, the solvent was removed. The brown oil was diluted the EtOAc (100 mL) and washed with H_2O (100 mL). The organic fraction was dried, filtered, and concentrated to a brown oil (7.79 g; 79%). The oil (3) (Scheme IV) was a mixture of methyl/ethyl ester products (9/1), and was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ_4.24 (q, J = 7.2 Hz, OCH_2), 3.91 (dd, 1H, J = 7.2, 7.0 Hz, CH), 3.62 (s, 3H, OCH_3), 3.42 (dd, 1H, J = 15.0, 7.1 Hz, 1 x CH_2); 3.02 (dd, 1H, J = 15.0, 7.0 Hz, 1 x CH_2); 2.44 (s, 3H, CH_3), 1.26 (t, J = 7.1 Hz, ester-CH_3).

[1]Seela, F.; Lüpke, U. Chem. Ber. 1977, 110, 1462-1469.

Preparation 2:

The procedure of Seela and Lüpke was used.[1] Thus, protection of the ketone (3) (Scheme IV; 5.0 g, 32.2 mmol) with ethylene glycol (4 mL, 64.4 mmol) in the presence of TsOH (100 mg) afforded (4) as an oil (Scheme IV; 5.2 g, 81.0) after flash chromatography (SiO_2; 3/7 EtOAc/Hex, R_f 0.35). Still contains ~5% ethyl ester: ^{1}H NMR (200 MHz, $CDCl_3$) δ_4.24 (q, J = 7.2 Hz, OCH_2), 3.98 (s, 4H, 2 x acetal-CH_2), 3.79 (s, 3H, OCH_3), 3.62 (dd, 1H, J = 7.2, 7.0 Hz, CH), 2.48 (dd, 1H, J = 15.0, 7.1 Hz, 1 x CH_2), 2.32 (dd, 1H, J = 15.0, 7.0 Hz, 1 x CH_2); 1.35 (s, 3H, CH_3), 1.26 (t, J = 7.1 Hz, ester-CH_3); MS (ES): 200.1 (M^++1).

[1]Seela, F.; Lüpke, U. Chem. Ber. 1977, 110, 1462-1469.

Preparation 3:

A solution of acetal (4) (Scheme IV, 1 g, 5.02 mmol), benzamidine (786 mg, 5.02 mmol), and DBU (1.5 mL, 10.04 mmol) in dry DMF (15 mL) was heated to 85°C for 15 h. The mixture was diluted with $CHCl_3$ (30 mL) and washed with 0.5 N NaOH (10 mL) and H_2O (20 mL). The organic fraction was dried, filtered and concentrated to a brown oil. Flash chromatography (SiO_2; 1/9 EtOAc/CH2Cl2, R_f 0.35) was attempted, but material crystallized on the column. The silica gel was washed with MeOH. Fractions containing the product (5) (Scheme IV) were concentrated and used without further purification (783 mg, 54.3%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.24 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 5.24 (br s, 2H, NH_2), 3.98 (s, 4H, 2 x acetal-CH_2), 3.60-3.15 (m, 2H, CH_2), 1.38 (s, 3H, CH_3); MS (ES): 288.1 (M^++1).

Preparation of compound (20) (Scheme VIII): A solution of acetal (19) (4.43 g, 20.6 mmol)[1], benzamine hydrochloride (3.22 g, 20.6 mmol), and DBU (6.15 mL, 41.2 mmol) in dry DMF (20 mL) was heated to 85°C for fifteen hours. The mixture was diluted with 100mL of $CHCl_3$, and washed with H_2O (2 x 50 mL). The organic fraction was dried, filtered, and concentrated to a dark brown oil. The dark brown oil was stirred in 1N HCl (100 mL) for 2 hours at room temperature. The resulting slurry was filtered yielding the HCl salt of (20) as a tan solid (3.60 g, 70.6%); ^{1}H NMR (200 MHz, DMSO-d6) 11.92 (s 1H), 8.05 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 7.05 (s, 1H, pyrrole-H); MS(ES): 212.1 (M^++1).

Preparation 4:

A solution of acetal (5) (700 mg, 2.44 mmol) in 1 N HCl (40 mL) was stirred for 2 h at RT. The resultant slurry was filtered yielding the HCl salt of 2-phenyl-6-methyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one as a tan solid (498 mg,

78.0%): ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.78 (s, 1H), 8.05 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 6.17 (s, 1H, pyrrole-H), 2.25 (s, 3H, CH_3); MS (ES): 226.1 (M^++1).

Preparation 5:

A modification of the Chen *et al.* cyclization method was used.[1] To an ice-cooled (0°C) solution of bromide (9), (Scheme V; 20.0 g, 108 mmol; 90% pure) in isopropyl alcohol (60 mL) was slowly added a solution of α-methylbenzylamine (12.5 mL, 97.3 mmol). The black solution was allowed to warm to RT and stir for 15 h. The mixture was diluted with EtOAc (200 mL) and washed with 0.5 N NaOH (50 mL). The organic fraction was dried, filtered, and concentrated to a black tar (19.2 g; 94%). The residue was partially purified by flash chromatography (SiO_2; 4/96 MeOH/CH_2Cl_2, R_f 0.35) to a black solid (6.38 g, 31%) as the compound *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4-methylpyrrole: MS (ES): 226.1 (M^++1).

[1]Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. *J. Med. Chem.* **1997**, 40, 1749-1754.

Preparation 6:

To a solution of *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4,5-dimethylpyrrole[1] (14.9 g, 62.5 mmol) and pyridine (10.0 mL) in dichloromethane (50.0 mL) was added benzoyl chloride (9.37 g, 66.7 mmol) at 0°C. After stirring at 0°C for 1 hr, hexane (10.0 mL) was added to help precipitation of product. Solvent was removed *in vacuo* and the solid was recrystallized from EtOH/H_2O to give 13.9 g (65%) of *dl*-1-(1-phenylethyl)-2-phenylcarbonylamino-3-cyano-4,5-dimethylpyrrole. mp 218-221°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.72 (s, 3H), 1.76 (d, J = 7.3 Hz, 3H), 1.98 (s, 3H), 5.52 (q, J = 7.3 Hz, 1H), 7.14-7.54 (m, 9H), 7.68-7.72 (dd, J = 1.4 Hz, 6.9 Hz , 2H), 10.73 (s, 1H);

MS (ES): 344.4 (M^++1).

[1] Liebigs Ann. Chem. 1986, 1485-1505.

The following compounds were obtained in a similar manner.

Preparation 6A:

dl-1-(1-phenylethyl)-2-(3-pyridyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.83 (d, J = 6.8 Hz, 3H), 2.02 (s, 3H), 2.12 (s, 3H), 5.50 (q, J = 6.8 Hz, 1H), 7.14-7.42 (m, 5H), 8.08 (m, 2H), 8.75 (m, 3H); MS (ES): 345.2 (M^++1).

dl-1-(1-phenylethyl)-2-(2-furyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.84 (d, J = 7.4 Hz, 3H), 1.92 (s, 3H), 2.09 (s, 3H), 5.49 (q, J = 7.4 Hz, 1H), 6.54 (dd, J = 1.8 Hz, 3.6 Hz, 1H), 7.12-7.47 (m, 7H); MS (ES): 334.2 (M^++1), 230.1.

dl-1-(1-phenylethyl)-2-(3-furyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.80 (d, J = 7 Hz 3H), 1.89 (s, 3H), 2.05 (s, 3H), 5.48 (q, J = 7 Hz, 1H), 6.59 (s, 1H), 7.12-7.40 (m, 6H), 7.93 (s, 1H); MS (ES): 334.1 (M^++1), 230.0.

dl-1-(1-phenylethyl)-2-cyclopentylcarbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.82 (d, J = 7.4 Hz, 3H), 1,88 (s, 3H), 2.05 (s, 3H), 1.63-1.85 (m, 8H), 2.63 (m, 1H), 5.43 (q, J = 7.4 Hz, 1H), 6.52 (s, 1H), 7.05-7.20 (m, 5H); MS (ES): 336.3 (M^++1).

dl-1-(1-phenylethyl)-2-(2-thienyl)carbonylamino-3-cyano-4,5-dimethylpyrrole, ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.82 (d, J = 6.8 Hz, 3H), 1.96 (s, 3H), 2.09 (s, 3H), 5.49 (q, J= 6.8 Hz, 1H), 7.05-7.55 (m, 8H); MS (ES): 350.1 (M^++1), 246.0.

dl-1-(1-phenylethyl)-2-(3-thienyl)carbonylamino-3-cyano-4,5-

dimethylpyrrole.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 7.0 Hz, 3H), 1.99 (s, 3H), 2.12 (s, 3H), 5.49 (q, J = 7.0 Hz, 1H), 6.90 (m, 1H), 7.18-7.36 (m, 6H), 7.79 (m, 1H); MS (ES): 350.2 (M^++1), 246.1.

dl-1-(1-phenylethyl)-2-(4-fluorophenyl)carbonylamino-3-cyano-4,5-dimethylpyrrole.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 7.4 Hz, 3H), 1.96 (s, 3H), 2.08 (s, 3H), 5.51 (q, J = 7.4 Hz, 1H), 7.16-7.55 (m, 9H); MS (ES): 362.2 (M^++1), 258.1.

dl-1-(1-phenylethyl)-2-(3-fluorophenyl)carbonylamino-3-cyano-4,5-dimethylpyrrole.

^{1}H NMR (200 MHz, $CDCl_3$) δ 1.83 (d, J = 7.4 Hz 3H), 1.97 (s, 3H), 2.10(s, 3H), 5.50 (q, J = 7.4 Hz, 1H), 7.05-7.38 (m, 7 H), 7.67-7.74 (m, 2H); MS (ES): 362.2 (M^++1), 258.1.

dl-1-(1-phenylethyl)-2-(2-fluorophenyl)carbonylamino-3-cyano-4,5-dimethylpyrrole. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85 (d, J = 7.2 Hz, 3H), 1.94 (s, 3H), 2.11 (s, 3H), 5.50 (q, J = 7.2 hz, 1H), 7.12-7.35 (m, 6H), 7.53 (m, 1H), 7.77 (m, 1H), 8.13 (m, 1H); MS (ES): 362.2(M^++1), 258.0.

dl-1-(1-phenylethyl)-2-isopropylcarbonylamino-3-cyano-4,5-dimethylpyrrole.^{1}H NMR (200 MHz, $CDCl_3$) δ 1.19 (d, J = 7.0 Hz, 6H), 1.82(d, J = 7.2 Hz, 3H), 1.88 (s, 3H), 2.06 (s, 3H), 2.46 (m, 1H), 5.39 (m, J = 7.2 Hz, 1H), 6.64 (s, 1H), 7.11-7.36 (m, 5H); MS (ES): 310.2 (M^++1), 206.1 .

In the case of acylation of *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4-methylpyrrole, monoacylated *dl*-1-(1-phenylethyl)-2-benzoylamino-3-cyano-4-dimethylpyrrole and diacylated pyrrole *dl*-1-(1-phenylethyl)-2-dibenzoylamino-3-cyano-4-methylpyrrole were obtained. Monoacylated pyrrole: ^{1}H NMR (200 MHz, $CDCl_3$) δ_7.69 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.58-7.12 (m, 8H, Ar-H),

6.18 (s, 1H, pyrrole-H), 5.52 (q, 1H, J = 7.2 Hz, C$\underline{H}$-CH_3), 2.05 (s, 3H, pyrrole-CH_3), 1.85 (d, 3H, J = 7.2 Hz, CH-C$\underline{H}_3$); MS (ES): 330.2 (M^++1); Diacylated pyrrole: ^{1}H NMR (200 MHz, $CDCl_3$) δ_7.85 (d, 2H, J = 7.7 Hz, Ar-H), 7.74 (d, 2H, J = 7.8 Hz, Ar-H), 7.52-7.20 (m, 9H, Ar-H), 7.04 (m, 2H, Ar-H), 6.21 (s, 1H, pyrrole-H), 5.52 (q, 1H, J = 7.2 Hz, C$\underline{H}$-CH_3), 1.77 (d, 3H, J = 7.2 Hz, CH-C$\underline{H}_3$), 1.74 (s, 3H, pyrrole-CH_3); MS (ES): 434.1 (M^++1).

Preparation 7:

To a solution of *dl*-1-(1-phenylethyl)-2-phenylcarboxyamido-3-cyano-4,5-dimethylpyrrole (1.0 g, 2.92 mmol) in methanol (10.0 mL) was added concentrated sulfuric acid (1.0 mL) at 0°C. The resulted mixture was refluxed for 15 hr and cooled down to room temperature. The precipitate was filtered to give 0.48 g (48%) of *dl*-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ_2.02 (d, J = 7.4 Hz, 3H), 2.04 (s, 3H), 2.41 (s, 3H), 6.25 (q, J = 7.4 Hz, 1H), 7.22-7.50 (m, 9H), 8.07-8.12 (dd, J = 3.4 Hz, 6.8 Hz, 2H), 10.51 (s, 1H); MS (ES): 344.2 (M^++1).

The following compounds were obtained in a similar manner as that of Preparation 7:

dl-5,6-dimethyl-2-(3-pyridyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ_2.03 (d, J = 7.2 Hz, 3H), 2.08 (s, 3H), 2.42 (s, 3H), 6.24 (q, J = 7.2 Hz, 1H), 7.09-7.42 (m, 5H), 8.48 (m, 2H), 8.70 (m, 3H); MS (ES): 345.1 (M^++1).

dl-5,6-dimethyl-2-(2-furyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.98 (d, J = 7.8 Hz, 3H), 1.99 (s, 3H), 2.37 (s, 3H), 6.12 (q, J = 7.8 Hz, 1H), 6.48 (dd, J=1.8 Hz, 3.6 Hz, 1H), 7.17-7.55 (m, 7H), 9.6 (s, 1H); MS (ES): 334.2 (M^++1).

dl-5,6-dimethyl-2-(3-furyl)-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.99 (d, J = 7 Hz, 3H), 2.02 (s, 3H), 2.42 (s, 3H), 6.24 (q, J = 7 Hz, 1H), 7.09 (s, 1H), 7.18-7.32 (m, 5H), 7.48 (s, 1H), 8.51 (s, 1H); MS (ES): 334.2 (M^++1).

dl-5,6-dimethyl-2-cyclopentyl-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.95 (d, J = 7.4 Hz, 3H), 2.00 (s, 3H), 2.33 (s, 3H), 1.68-1.88 (m, 8H), 2.97 (m, 1H), 6.10 (q, J = 7.4 Hz, 1H), 7.16-7.30 (m, 5H), 9.29 (s, 1H); MS (ES): 336.3 (M^++1).

dl-5,6-dimethyl-2-(2-thienyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02(d, J = 7.2 Hz, 3H), 2.06 (s, 3H), 2.41 (s, 3H), 6.13 (q, J = 7.2 Hz, 1H), 7.12 (dd, J = 4.8, 2.8 Hz, 1H), 7.26-7.32 (m, 5H), 7.44 (d, J = 4.8 Hz, 1H), 8.01 (d, J = 2.8 Hz, 1H) 11.25 (s, 1H); MS (ES): 350.2 (M^++1).

dl-5,6-dimethyl-2-(3-thienyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.00 (d, J = 7.4 Hz, 3H), 2.05 (s, 3H), 2.43 (s, 3H), 6.24(q, J = 7.4 Hz, 1H), 7.24-7.33 (m, 5H), 7.33-7.39 (m, 1H), 7.85 (m, 1H), 8.47 (m, 1H), 12.01 (s, 1H); MS (ES): 350.2 (M^++1).

dl-5,6-dimethyl-2-(4-fluorophenyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.01 (d, J = 6.8 Hz, 3H), 2.05 (s, 3H), 2.42 (s, 3H), 6.26 (q, J = 6.8 Hz, 1H), 7.12-7.36 (m, 7H), 8.23-8.30 (m, 2H), 11.82 (s, 1H); MS (ES): 362.3 (M^++1).

dl-5,6-dimethyl-2-(3-fluorophenyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02 (d, J = 7.4 Hz, 3H), 2.06 (s, 3H), 2.44 (s, 3H), 6.29 (q,

J = 7.4 Hz, 1H), 7.13-7.51(m, 7H), 8.00-8.04 (m, 2H), 11.72 (s, 1H); MS (ES): 362.2 (M^++1).

dl-5,6-dimethyl-2-(2-fluorophenyl)-7*H*-7-(1-phenylethyl) pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^1H NMR (200 MHz, $CDCl_3$) δ 2.00(d, J = 7.2 Hz, 3H), 2.05 (s, 3H), 2.38 (s, 3H), 6.24 (q, J = 7.2 Hz, 1H), 7.18 - 7.45 (m, 8 H), 8.21 (m, 1H), 9.54 (s, 1H); MS (ES): 362.2 (M^++1).

dl-5,6-dimethyl-2-isopropyl-7*H*-7-(1-phenylethyl)pyrrolo [2,3d]pyrimidin-4(3*H*)-one.
^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (d, J = 6.8 Hz, 3H), 1.32 (d, J = 7.0 Hz, 3H), 2.01 (s, 3H), 2.34 (s, 3H), 2.90 (m, 1H), 6.13 (m, 1H), 7.17-7.34 (m, 5H), 10.16 (s, 1H); MS (ES): 310.2 (M^++1).

Preparation 8:

A solution of *dl*-1-(1-phenylethyl)-2-benzoylamino-3-cyano-4-methylpyrrole (785 mg, 2.38 mmol) with concentrated H_2SO_4 (1 mL) in DMF (13 mL) was stirred at 130°C for 48 h. The black solution was diluted with $CHCl_3$ (100 mL) and washed with 1 N NaOH (30 mL), and brine (30 mL). The organic fraction was dried, filtered, concentrated, and purified by flash chromatography (SiO_2; 8/2 EtOAc/Hex, R_f 0.35) to a brown solid (184 mg, 24%) as *dl*-5-methyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^1H NMR (200 MHz, $CDCl_3$) δ_8.18 (m, 2H, Ar-H), 7.62-7.44 (m, 3H, Ar-H), 7.40-7.18 (m, 5H, Ar-H), 6.48 (s, 1H, pyrrole-H), 6.28 (q, 1H, J = 7.2 Hz, C$\underline{H}$-CH_3), 2.18 (s, 3H, pyrrole-CH_3), 2.07 (d, 3H, J = 7.2 Hz, CH-C$\underline{H}_3$); MS (ES): 330.2 (M^+ + 1).

Preparation 9:

A mixture of *dl*-1-(1-phenylethyl)-2-amino-3-cyano-4,5-dimethylpyrrole (9.60 g, 40.0 mmol) and of formic acid (50.0

mL, 98%) was refluxed for 5 hr. After cooling down to room temperature and scratching the sides of flask, copious precipitate was formed and filtered. The material was washed with water until washings showed neutral pH to give *dl*-5,6-dimethyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.96 (d, J = 7.4 hz, 3H), 2.00 (s, 3H), 2.38 (s, 3H), 6.21 (q, J = 7.4 Hz, 1H), 7.11-7.35 (m, 5H), 7.81 (s, 1H), 11.71 (s, 1H); MS (ES): 268.2 (M^++1).

Preparation 10:

dl-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl) pyrrolo [2,3d]pyrimidin-4(3*H*)-one (1.0 g, 2.91 mmol) was suspended in polyphosphoric acid (30.0 mL). The mixture was heated at 100°C for 4 hr. The hot suspension was poured onto ice water, stirred vigorously to disperse suspension, and basified to pH 6 with solid KOH. The resulting solid was filtered and collected to give 0.49 g (69%) of 5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ_2.17 (s, 3H), 2.22 (s, 3H), 7.45 (br, 3H), 8.07 (br, 2H,), 11.49 (s, 1H), 11.82 (s, 1H); MS (ES): 344.2 (M^++1).

The following compounds were obtained in a similar manner as that of Preparation 10:

5-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. MS (ES): 226.0 (M^++1).

5,6-dimethyl-2-(3-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. MS (ES): 241.1 (M^++1).

5,6-dimethyl-2-(2-furyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.13 (s, 3H), 2.18 (s, 3H), 6.39 (dd, J = 1.8, 3.6 Hz, 1H), 6.65 (dd, J = 1.8 Hz, 3.6 Hz, 1H), 7.85 (dd, J = 1.8, 3.6 Hz, 1H,), 11.45 (s, 1H), 11.60 (s, 1H);

MS (ES): 230.1 (M^++1).

5,6-dimethyl-2-(3-furyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.19 (s, 3H), 6.66 (s, 1H), 7.78 (s, 1H), 8.35 (s, 1H), 11.3 (s, 1H), 11.4 (s, 1H); MS (ES): 230.1 (M^++1).

5,6-dimethyl-2-cyclopentyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.57-1.91 (m, 8 H), 2.12 (s, 3H), 2.16 (s, 3H), 2.99 (m, 1H), 11.24 (s, 1H), 11.38 (s, 1H); MS (ES): 232.2 (M^++1).

5,6-dimethyl-2-(2-thienyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.14 (s, 3H), 2.19 (s, 3H), 7.14 (dd, J = 3.0, 5.2 Hz, 1H), 7.70 (d, J = 5.2 Hz 1H), 8.10 (d, J=3.0 Hz, 1H), 11.50 (s, 1H); MS (ES): 246.1 (M^++1).

5,6-dimethyl-2-(3-thienyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.17 (s, 3H), 2.21(s, 3H), 7.66(m, 1H), 7.75 (m, 1H), 8.43 (m, 1H), 11.47 (s, 1H), 11.69 (s, 1H); MS (ES): 246.1 (M^++1).

5,6-dimethyl-2-(4-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.17 (s, 3H), 2.21 (s, 3H), 7.31 (m, 2H), 8.12 (m, 2H), 11.47 (s, 1H); MS (ES): 258.2 (M^++1).

5,6-dimethyl-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.21 (s, 3H), 7.33 (m, 1H), 7.52 (m, 1H), 7.85-7.95 (m, 2H), 11.56 (s, 1H), 11.80 (s, 1H); MS (ES): 258.1 (M^++1).

5,6-dimethyl-2-(2-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.18 (s, 3H), 2.22 (s,

3H), 7.27-7.37 (m, 2H), 7.53 (m 1H), 7.68 (m, 1H), 11.54 (s, 1H), 11.78 (s, 1H); MS (ES): 258.1 (M^{+}+1).

5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.17 (d, J= 6.6 Hz, 6H), 2.11 (s, 3H), 2.15 (s, 3H), 2.81 (m, 1H), 11.20 (s, 1H), 11.39 (s, 1H); MS (ES): 206.1 (M^{+}+1).

5,6-dimethyl-7*H*-pyrrolo[2,3d]pyrimidin-4(3*H*)-one. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.13 (s, 3H), 2.17 (s, 3H), 7.65 (s, 1H); MS (ES): 164.0 (M^{+}+1).

Preparation 11:

A solution of 5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidin-4(3*H*)-one (1.0 g, 4.2 mmol) in phosphorus oxychloride (25.0 mL) was refluxed for 6 hr and then concentrated *in vacuo* to dryness. Water was added to the residue to induce crystallization and the resulting solid was filtered and collected to give 0.90 g (83%) of 4-chloro-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ_2.33 (s, 3H), 2.33 (s, 3H), 7.46-7.49 (m, 3H), 8.30-8.35 (m, 2H), 12.20 (s, 1H); MS (ES): 258.1 (M^{+}+1).

The following compounds were obtained in a similar manner as that of Preparation 11:

4-chloro-5-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 244.0 (M^{+}+1).

4-chloro-6-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 244.0 (M^{+}+1).

4-chloro-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d6) 8.35 (2, 2H), 7.63 (br s, 1H), 7.45 (m, 3H), 6.47 (br s, 1H); MS (ES): 230.0 (M^{+}+1).

4-chloro-5,6-dimethyl-2-(3-pyridyl)-7*H*-pyrrolo[2,3d] pyrimidine. MS (ES): 259.0 (M^{+}+1).

4-chloro-5,6-dimethyl-2-(2-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.35 (s, 3H), 2.35 (s, 3H), 6.68 (dd, J = 1.8, 3.6 Hz, 1H), 7.34 (dd, J = 1.8 Hz, 3.6 Hz, 1H), 7.89 (dd, J = 1.8, 3.6 Hz, 1H); MS (ES): 248.0 (M^{+}+1).

4-chloro-5,6-dimethyl-2-(3-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31 (s, 3H), 2.31 (s, 3H), 6.62 (s, 1H), 7.78 (s, 1H), 8.18 (s, 1H), 12.02 (s, 1H); MS (ES): 248.1 (M^{+}+1).

4-chloro-5,6-dimethyl-2-cyclopentyl-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.61- 1.96 (m, 8H), 2.27 (s, 3H), 2.27 (s, 3H), 3.22 (m, 1H), 11.97 (s, 1H); MS (ES): 250.1 (M^{+}+1).

4-chloro-5,6-dimethyl-2-(2-thienyl)-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.29 (s, 3H), 2.31 (s, 3H), 7.14 (dd, J = 3.1 Hz, 4.0 Hz, 1H), 7.33 (d, J = 4.9 Hz, 1H), 7.82 (d, J = 3.1 Hz, 1H), 12.19 (s, 1H); MS (ES): 264.1 (M^{+}+1).

4-chloro-5,6-dimethyl-2-(3-thienyl)-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.32 (s, 3H), 2.32 (s, 3H), 7.62 (dd, J = 3.0, 5.2 Hz, 1H), 7.75 (d, J = 5.2 Hz, 1H), 8.20 (d, J = 3.0 Hz, 1H); MS (ES): 264.0 (M^{+}+1).

4-chloro-5,6-dimethyl-2-(4-fluorophenyl)-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.33(s, 3H), 2.33 (s, 3H), 7.30 (m, 2H), 8.34 (m, 2H), 12.11 (s, 1H); MS (ES): 276.1. (M^{+}+1).

4-chloro-5,6-dimethyl-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]

pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31(s, 3H), 2.33 (s, 3H), 7.29(m, 1H), 7.52 (m, 1H), 7.96 (m, 1H), 8.14(m, 1H), 11.57 (s, 1H); MS (ES): 276.1 (M^++1).

4-chloro-5,6-dimethyl-2-(2-fluorophenyl)-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.34 (s, 3H), 2.34 (s, 3H), 7.33 (m, 2H), 7.44 (m, 1H), 7.99 (m, 1H), 12.23 (s, 1H); MS (ES): 276.1 (M^++1).

4-chloro-5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.24 (d, J = 6.6 Hz, 6H), 2.28 (s, 3H), 2.28 (s, 3H), 3.08 (q, J = 6.6 Hz, 1H), 11.95 (s, 1H); MS (ES): 224.0 (M^++1).

4-chloro-5,6-dimethyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.31 (s, 3H), 2.32 (s, 3H), 8.40 (s, 1H); MS (ES): 182.0 (M^++1).

dl-4-chloro-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo [2,3d]pyrimidine.

Preparation 12:

To a solution of *dl*-1,2-diaminopropane (1.48 g, 20.0 mmol) and sodium carbonate (2.73 g, 22.0 mmol) in dioxane (100.0 mL) and water (100.0 mL) was added di-*tert*-butyldicarbonate (4.80 g, 22.0 mmol) at room temperature. The resulted mixture was stirred for 14 hr. Dioxane was removed *in vacuo*. The precipitate was filtered off and the filtrate was concentrated *in vacuo* to dryness. The residue was triturated with EtOAc and then filtered. The filtrate was concentrated *in vacuo* to dryness to give a mixture of *dl*-1-amino-2-(1,1-dimethylethoxy)carbonylamino-propane and *dl*-2-amino-1-(1,1-dimethylethoxy)carbonylamino-propane which were not separable by normal chromatography method. The mixture was used for the

reaction in Example 8.

Preparation 13:

To solution of Fmoc-β-Ala-OH (1.0 g, 3.212 mmol) and oxalyl chloride (0.428 g, 0.29 mL, 3.373 mmol) in dichloromethane (20.0 mL) was added a few drops of N,N-dimethylformamide at 0°C. The mixture was stirred at room temperature for 1 hr followed by addition of cyclopropylmethylamine (0.229 g, 0.28 mL, 3.212 mmol) and triethylamine (0.65 g, 0.90 mL, 6.424 mmol). After 10 min, the mixture was treated with 1 M hydrochloride (10.0 mL) and the aqueous mixture was extracted with dichloromethane (3 x 30.0 mL). The organic solution was concentrated *in vacuo* to dryness. The residue was treated with a solution of 20% piperidine in N,N-dimethylforamide (20.0 mL) for 0.5 hr. After removal of the solvent *in vacuo,* the residue was treated with 1 M hydrochloride (20.0 mL) and ethyl acetate (20.0 mL). The mixture was separated and the aqueous layer was basified with solid sodium hydroxide to pH = 8. The precipitate was removed by filtration and the aqueous solution was subjected to ion exchange column eluted with 20% pyridine to give 0.262 g (57%) of N-cyclopropylmethyl β-alanine amide. ^{1}H NMR (200 MHz, CD_3OD) δ_0.22 (m, 2H), 0.49 (m, 2H), 0.96 (m, 2H), 2.40 (t, 2H), 2.92 (t, 2H), 3.05 (d, 2H); MS (ES): 143.1 (M^++1).

Preparation 14:

N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine.

trans-1,4-cyclohexyldiamine (6.08 g, 53.2 mmol) was dissolved in dichloromethane (100mL). A solution of di-t-butyldicarbonate (2.32 g, 10.65 mmol in 40 mL dichloromethane) was added via cannula. After 20 hours, the reaction was partitioned between $CHCl_3$ and water. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to yield 1.20 g of a white solid (53%). ^{1}H-

NMR (200MHz, $CDCl_3$): δ 1.0-1.3 (m, 4H), 1.44 (s, 9H), 1.8 -2.1 (m, 4H), 2.62 (brm, 1H), 3.40 (brs, 1H), 4.37 (brs, 1H0; MS (ES): 215.2 (M^++1).

4-(N-acetyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyl diamine.

N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine (530 mg, 2.47 mmol) was dissolved in dichloromethane (20 mL). Acetic anhydride (250 mg, 2.60 mmol) was added dropwise. After 16 hours, the reaction was diluted with water and $CHCl_3$. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Recrystallization (EtOH/H_2O) yielded 190 mg of white crystals (30%). ^1H NMR (200 MHz, $CDCl_3$): δ 0.9 - 1.30 (m, 4H), 1.43 (s, 9H), 1.96-2.10 (m, 7H), 3.40 (brs, 1H), 3.70 (brs, 1H), 4.40 (brs, 1H), 4.40 (brs, 1H); MS (ES): 257.2 (M^++1), 242.1 (M^+ - 15), 201.1 (M^+ - 56).

4-(4-*trans*-acetamidocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-(1-phenylethyl) pyrrolo[2,3d]pyrimidine.

4-(N-acetyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine (190 mg, 0.74 mmol), was dissolved in dichloromethane (5 mL) and diluted with TFA (6 ml). After 16 hours, the reaction was concentrated. The crude solid, DMSO (2mL), $NaHCO_3$ (200 mg, 2.2 mmol) and 4-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine (35 mg, 0.14 mmol) were combined in a flask and heated to 130 °C. After 4.5 hours, the reaction was cooled to room temperature and diluted with EtOAc and water. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Chromatography (silica preparatory plate; 20:1 $CHCl_3$:EtOH) yielded 0.3 mg of a tan solid (1% yield). MS (ES): 378.2 (M^++1).

4-(N-methanesulfonyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-

cyclohexyldiamine.

trans-1,4-cyclohexyldiamine (530 mg, 2.47 mmol) was dissolved in dichloromethane (20 ml) and diluted with pyridine (233 mg, 3.0 mmol). Methanesulfonyl chloride (300 mg, 2.60 mmol) was added dropwise. After 16 hours, the reaction was diluted with water and $CHCl_3$. The layers were separated and the aqueous layer was extracted with $CHCl_3$ (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. recrystallization ($EtOH/H_2O$) yielded 206 mg of white crystals (29%). ^{1}H-NMR (200MHz, $CDCl_3$): δ 1.10-1.40 (m, 4H), 1.45 (s, 9H), 2.00-2.20 (m, 4H), 2.98 (s, 3H), 3.20-3.50 (brs, 2H), 4.37 (brs, 1H); MS (ES) 293.1 (M^++1), 278.1 (M^+-15), 237.1 (M^+-56).

4-(4-*trans*-methanesulfamidocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(N-sulfonyl)-N-*tert*-butoxycarbonyl-*trans*-1,4-cyclohexyldiamine (206 mg, 0.71 mmol), was dissolved in dichloromethane (5ml) and diluted with TFA (6 ml). After 16 hours, the reaction was concentrated. The crude reaction mixture, DMSO (2 ml), $NaHCO_3$ (100 mg, 1.1 mmol) and 1-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine were combined in a flask and heated to 130 °C. After 15 hours, the reaction was cooled to room temperature, and diluted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Chromatography (silica preparatory plate, 20:1 $CHCl_3$/EtOH) yielded 2.6 mg of a tan solid (5% yield). MS (ES): 414.2 (M^++1).

Example 1:

A solution of 4-chloro-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine (0.50 g, 1.94 mmol) and 4-*trans*-hydroxy cyclohexylamine (2.23 g, 19.4 mmol) in methyl sulfoxide (10.0 mL) was heated at 130°C for 5 hr. After cooling down to room temperature, water (10.0 mL) was added and the resulted aqueous solution was extracted with EtOAc (3 x10.0 mL). The combined EtOAc solution was dried ($MgSO_4$) and filtered, the filtrate was concentrated *in vacuo* to dryness, the residue was chromatographed on silica gel to give 0.49 g (75%) of 4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. mp 197-199°C; ^1H NMR (200 MHz, $CDCl_3$) δ_1.25-1.59 (m, 8H), 2.08 (s, 3H), 2.29 (s, 3H), 3.68-3.79 (m, 1H), 4.32-4.38 (m, 1H), 4.88 (d, J = 8 Hz, 1H), 7.26-7.49 (m, 3H), 8.40-8.44 (dd, J = 2.2, 8 Hz, 2H), 10.60 (s, 1H); MS (ES): 337.2 (M^++1).

The following compounds were obtained in a similar manner to that of Example 1:

4-(4-*trans*-hydroxycyclohexyl)amino-6-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ_11.37 (s, 1H, pyrrole-NH), 8.45 (m, 2H, Ar-H), 7.55 (m, 3H, Ar-H), 6.17 (s, 1H, pyrrole-H), 4.90 (br d, 1H, NH), 4.18 (m, 1H, CH-O), 3.69 (m, 1H, CH-N), 2.40-2.20 (m, 2H), 2.19-1.98 (m, 2H), 2.25 (s, 3H, CH3) 1.68-1.20 (m, 4H); MS (ES): 323.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5-methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ_11.37 (s, 1H, pyrrole-NH), 8.40 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 5.96 (s, 1H, pyrrole-H), 4.90 (br d, 1H, NH), 4.18 (m, 1H, CH-O), 3.69 (m, 1H, CH-N), 2.38-2.20 (m, 2H), 2.18-1.98 (m, 2H), 2.00 (s, 3H, CH3) 1.68-1.20 (m, 4H); MS (ES): 323.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-2-phenyl-7*H*-pyrrolo[2,3d]

pyrimidine. mp 245.5-246.5°C; ^{1}H NMR (200MHz, CD_3OD) δ 8.33(m, 2H, Ar-H), 7.42 (m, 3H, Ar-H), 7.02 (d, 1H, J=3.6 Hz, pyrolle-H), 6.53 (d, 1H, J=3.6 Hz, pyrolle-H), 4.26 (m, 1H, CH-O), 3.62 (m,1H, CH-N), 2.30-2.12 (m, 2H), 2.12-1.96 (m, 2H), 1.64-1.34 (m, 4H); MS, M+1=309.3; Anal ($C_{18}H_{20}N_4O$) C, H, N.

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.21-1.54 (m, 8H); 2.28 (s, 3H); 2.33 (s, 3H); 3.70 (m, 1H), 4.31(m, 1H), 4.89 (d, 1H), 7.40 (m, 1H), 8.61 (m, 2H), 9.64 (m, 1H); MS (ES): 338.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(2-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-1.64(m, 8H), 2.22 (s, 3H), 2.30 (s, 3H), 3.72(m, 1H), 4.23 (m, 1H), 4.85 (d, 1H), 6.52(m, 1H), 7.12 (m, 1H), 7.53 (m, 1H), 9.28 (s, 1H); MS (ES): 327.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-furyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25-1.63 (m, 8 H), 2.11 (s, 3H), 2.27 (s, 3H), 3.71(m, 1H), 4.20 (m, 1H), 4.84 (d, 1H), 7.03 (m, 1H), 7.45(m, 1H), 8.13(m, 1H), 10.38 (m, 1H); MS (ES): 327.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-cyclopentyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-2.04 (m, 16 H), 2.26 (s, 3H), 2.27 (s, 3H), 3.15(m, 1H), 3.70 (m, 1H), 4.12 (m, 1H), 4.75(d, 1H); MS (ES): 329.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(2-thienyl)-7*H*-pyrrolo[2,3d]pyrimidin-4-amine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28-1.59 (m, 8H), 2.19 (s, 3H), 2.29 (s, 3H), 3.74 (m, 1H), 4.19 (m, 1H), 4.84 (d, 1H), 7.09 (m, 1H), 7.34 (m, 1H), 7.85 (m, 1H), 9.02 (s, 1H); MS (ES): 343.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-thienyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21-1.60 (m, 8H), 1.98 (s, 3H), 2.23 (s, 3H), 3.66 (m, 1H), 4.22 (m, 1H), 7.27 (m, 1H), 7.86 (m, 1H), 8.09 (m, 1H), 11.23 (s, 1H); MS (ES): 343.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(4-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26- 1.66 (m, 8H), 1.94 (s, 3H), 2.28 (s, 3H), 3.73 (m, 1H), 4.33 (m, 1H), 4.92 (d, 1H), 7.13 (m, 2H), 8.41 (m, 2H), 11.14 (s, 1H); MS (ES): 355.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(3-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-1.71 (m, 8H), 2.06 (s, 3H), 2.30 (s, 3H), 3.72 (m, 1H), 4.30 (m, 1H), 4.90 (d, 1H), 7.09 (m, 1H), 7.39 (m, 1H), 8.05 (m, 1H), 8.20 (m, 1H), 10.04 (s. 1H); MS (ES): 355.2 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-(2-fluorophenyl)-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30-1.64 (m, 8H), 2.17 (s, 3H), 2.31 (s, 3H), 3.73 (m, 1H), 4.24 (m, 1H), 4.82 (d, 1H), 7.28 (m, 2H), 8.18 (m, 1H), 9.02 (m, 1H), 12.20 (s, 1H); MS (ES): 355.3 (M^++1).

4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidine ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, J = 7.0 Hz, 6H), 1.30-1.65 (m, 8H), 2.27 (s, 3H), 2.28 (s, 3H), 3.01 (m, J = 7.0 Hz, 1H), 3.71 (m, 1H), 4.14 (m, 1H), 4.78 (d, 1H); MS (ES): 303.2.

dl-4-(2-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-isopropyl-7*H*-pyrrolo[2,3d]pyrimidine ^{1}H NMR (200 MHz, $CDCl_3$) d 1.31-1.42 (br, 4H), 1.75-1.82 (br, 4H), 2.02 (s, 3H), 2.29 (s, 3H), 3.53 (m, 1H), 4.02 (m, 1H), 5.08 (d, 1H), 7.41-7.48

(m, 3H), 8.30 (m, 2H), 10.08 (s, 1H); MS (ES): 337.2 (M^{+}+1).

4-(3,4-*trans*-dihydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^{+}+1).

4-(3,4-*cis*-dihydroxylcyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^{+}+1).

4-(2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.
mp 196-199°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.72 (s, 3H), 1.97 (s, 3H), 2.31 (s, 3H), 3.59 (m, 2H), 3.96 (m, 2H), 5.63 (br, 1H), 7.44-7.47 (m, 3H), 8.36-8.43 (dd, J = 1 Hz, 7 Hz, 2H), 10.76 (s, 1H); MS (ES): 324.5 (M^{+}+1).

dl-4-(2-*trans*-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1]

^{1}H NMR (200 MHz, $CDCl_3$) δ_1.62 (m, 2H), 1.79 (br, 4H), 1.92 (s, 3H), 2.29 (s, 3H), 4.11 (m, 1H), 4.23 (m, 1H), 5.28 (d, 1H), 7.41-7.49 (m, 3H), 8.22 (m, 2H), 10.51 (s, 1H); MS (ES): 323.2 (M^{+}+1).

[1] For preparation of 2-*trans*-hydroxycyclopentylamine, see PCT 9417090.

dl-4-(3-*trans*-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1]
^{1}H NMR (200 MHz, $CDCl_3$) δ_1.58-1.90 (br, 6 H,), 2.05 (s, 3H), 2.29 (s, 3H), 4.48-4.57 (m, 1H), 4.91-5.01 (m, 2H), 7.35-7.46 (m, 3H), 8.42-8.47 (m, 2H), 10.11 (s, 1H); MS (ES): 323.2 (M^{+}+1).

[1] For preparation of 3-*trans*-hydroxycyclopentylamine, see EP-A-322242.

dl-4-(3-*cis*-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.[1]
^{1}H NMR (200 MHz, $CDCl_3$) δ_1.82-2.28 (br, 6H), 2.02 (s, 3H), 2.30 (s, 3H), 4.53-4.60 (m, 1H), 4.95-5.08 (m, 1H), 5.85-5.93 (d, 1H), 7.35-7.47 (m, 3H), 8.42-8.46 (m, 2H), 10.05 (s, 1H); MS (ES): 323.2 (M^++1).
[1] For preparation of 3-*cis*-hydroxycyclopentylamine, see EP-A-322242.

4-(3,4-*trans*-dihydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl -7*H*-pyrrolo[2,3d]pyrimidine.[1] ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.92-1.99 (br, 2H), 2.14 (s, 3H), 2.20 (br, 2H), 2.30 (s, 3H), 2.41-2.52 (br, 2H), 4.35 (m, 2H), 4.98 (m, 2H), 7.38-7.47 (m, 3H), 8.38-8.42 (m, 2H), 9.53 (s, 1H); MS (ES): 339.2 (M^++1).
[1] For preparation of 3,4-*trans*-dihydroxycyclopentylamine, see PCT 9417090.

4-(3-amino-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.
^{1}H NMR (200 MHz, $CDCl_3$) δ_2.02 (s, 3H), 2.29 (s, 3H), 2.71 (t, 2H), 4.18 (m, 2H), 5.75-5.95 (m, 3H), 7.38-7.48 (m, 3H), 8.37-8.41 (m, 2H), 10.42 (s, 1H); MS (ES): 310.1 (M^++1).

4-(3-N-cyclopropylmethylamino-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, CD_3OD) δ_0.51 (q, 2H), 0.40 (q, 2H), 1.79-1.95 (br, 1H), 2.36 (s, 3H), 2.40 (s, 3H), 2.72 (t, 2H), 2.99 (d, 2H), 4.04 (t, 2H), 7.58-7.62 (m, 3H), 8.22-8.29 (m, 2H); MS (ES): 364.2 (M^++1).

4-(2-amino-2-oxoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine ^{1}H NMR (200 MHz, CD_3OD) δ 2.31 (s, 3H), 2.38 (s, 3H), 4.26 (s, 2H), 7.36 (m, 3H), 8.33 (m, 2H); MS (ES): 396.1 (M^++1).

4-(2-N-methylamino-2-oxoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-

pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.99 (s, 3H), 2.17 (s, 3H), 2.82 (d, 3H), 4.39 (d, 2H), 5.76 (t, 1H), 6.71 (br, 1H), 7.41-7.48 (m, 3H), 8.40 (m, 2H), 10.66 (s, 1H); MS (ES): 310.1 (M^++1).

4-(3-*tert*-butyloxyl-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.45 (s, 9H), 1.96 (s, 3H), 2.29 (s, 3H), 2.71 (t, 2H), 4.01 (q, 2H), 5.78 (t, 1H), 7.41-7.48 (m, 3H), 8.22-8.29 (m, 2H); MS (ES): 367.2 (M^++1).

4-(2-hydroxyethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.92 (s, 3H), 2.29 (s, 3H), 3.81-3.98 (br, 4H), 5.59 (t, 1H), 7.39-7.48 (m, 3H), 8.37 (m, 2H), 10.72 (s, 1H); MS (ES): 283.1 (M^++1).

4-(3-hydroxypropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.84 (m, 2H), 1.99 (s, 3H), 2.32 (s, 3H), 3.62 (t, 2H), 3.96 (m, 2H), 3.35 (t, 1H), 7.39-7.48 (m, 3H), 8.36 (m, 2H), 10.27 (s, 1H); MS (ES): 297.2 (M^++1).

4-(4-hydroxybutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.71-1.82 (m, 4H), 1.99 (s, 3H), 2.31 (s, 3H), 3.68-3.80 (m, 4H), 5.20 (t, 1H), 7.41-7.49 (m, 3H), 8.41(m, 2H), 10.37 (s, 1H); MS (ES): 311.2 (M^++1).

4-(4-*trans*-acetylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

4-(4-*trans*-methylsulfonylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.

4-(2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-

phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(4-*trans*-hydoxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(3-pyridylmethyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

4-(2-methylpropyl)amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3d]pyrimidine.

Example 2:

To a stirred suspension of triphenylphosphine (0.047 g, 0.179 mmol) and benzoic acid (0.022 g, 0.179 mmol) in THF (1.0 mL) cooled to 0°C was added 4-(4-*trans*-hydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (0.05 g, 0.149 mmol) at 0°C. Diethyl azodicarboxylate (0.028 ml, 0.179 mmol) was then added dropwise over 10 minutes. The reaction was then allowed to warm to room temperature. After reaction was complete by TLC the reaction mixture was quenched with aqueous sodium bicarbonate (3.0 mL). The aqueous phase was separated and extracted with ether (2 X 5.0 mL). The organic extracts were combined, dried, and concentrated *in vacuo* to dryness. To the residue was added ether (2.0 mL) and hexane (5.0 mL) whereupon the bulk of the triphenylphosphine oxide was filtered off. Concentration of the filtrate gave a viscous oil which was purified by column chromatography (hexane:ethyl acetate=4:1) to give 5.0 mg (7.6%) of 4-(4-*cis*-benzoyloxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 441.3 (M^{+}+1). The reaction also produced 50.0 mg (84%) of 4-(3-cyclohexenyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 319.2 (M^{+}+1).

Example 3:

To a solution of 4-(4-*cis*-benzoyloxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (5.0 mg, 0.0114 mmol) in ethanol (1.0 mL) was added 10 drops of 2M sodium hydroxide. After 1 hr, the reaction mixture was extracted with ethyl acetate (3 x 5.0 mL) and the organic layer was dried, filtered and concentrated *in vacuo* to dryness. The residue was subjected to column chromatography (hexane:ethyl acetate=4:1) to give 3.6 mg (94%) of 4-(4-*cis*-hydroxycyclohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 337.2 (M^{+}+1).

The following compounds were obtained in a similar manner as that of Example 3:

4-(3-N,N-dimethyl-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.31 (s, 3H), 2.73 (t, 2H), 2.97 (s, 6H), 4.08 (m, 2H), 6.09 (t, 1H), 7.41-7.48 (m, 3H), 8.43 (m, 2H), 10.46 (s, 1H); MS (ES): 338.2 (M^{+}+1).

4-(2-formylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.26 (s, 3H), 2.37 (s, 3H), 3.59-3.78 (m, 2H), 3.88-4.01 (m, 2H), 5.48-5.60 (m, 1H), 7.38-7.57 (m, 3H), 8.09 (s, 1H), 8.30-8.45 (m, 2H), 8.82 (s, 1H); MS (ES): 310.1 (M^{+}+1).

4-(3-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 338.2 (M^{+}+1).

Example 4:

4-(3-*tert*-butyloxy-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (70.0 mg, 0.191 mmol)) was dissolved in trifluoroacetic acid:dichloromethane (1:1, 5.0 mL). The resulting solution was stirred at room temperature for 1 hr. and then refluxed for 2 hr. After cooling down to room temperature, the mixture was concentrated *in vacuo* to dryness. The residue was subjected to preparative thin layer chromatography (EtOAc:hexane: AcOH=7:2.5:0.5) to give 40.0 mg (68%) of. 4-(3-hydroxy-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, CD_3OD) δ 2.32 (s, 3H), 2.38 (s, 3H), 2.81 (t, 2H), 4.01 (t, 2H), 7.55 (m, 3H), 8.24 (m, 2H); MS (ES): 311.1 (M^++1).

The following compound was obtained in a similar manner as that of Example 4:

4-(3-aminopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 296.1 (M^++1), 279.1 (M^+-NH_3).

Example 5:

4-(3-hydroxy-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (50.0 mg, 0.161 mmol) was dissolved in a mixture of N,N-dimethylformamide (0.50 mL), dioxane (0.50 mL) and water(0.25 mL). To this solution was added methylamine (0.02 mL, 40% wt in water, 0.242 mmol), triethylamine (0.085 mL) and N,N,N'N'-tetramethyl uronium tetrafluoroborate (61.2 mg, 0.203 mmol). After stirring at room temperature for 10 min, the solution was concentrated and the residue was subjected to preparative thin layer chromatography (EtOAc) to give 35.0 mg (67%) of 4-(3-N-methyl-3-oxopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.92 (s, 3H), 2.30 (s, 3H), 2.65 (t, 2H), 4.08 (t, 2H), 5.90 (t, 1H), 6.12 (m, 1H), 7.45 (m, 3H), 8.41 (m, 2H),

10.68 (s, 1H); MS (ES): 311.1 (M^{+}+1).

The following compounds were obtained in a similar manner as that of Example 5:

4-(2-cyclopropanecarbonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 350.2 (M^{+}+1).

4-(2-isobutyrylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 352.2 (M^{+}+1).

4-(3-propionylaminopropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00-1.08 (t, 3H), 1.71-2.03 (m, 4H), 2.08 (s, 3H), 2.37 (s, 3H), 3.26-3.40 (m, 2H), 3.79-3.96 (m, 2H), 5.53-5.62 (m, 1H), 6.17-6.33 (m, 1H), 7.33-7.57 (m, 3H), 8.31-8.39 (m, 2H), 9.69 (s, 1H); MS (ES): 352.2 (M^{+}+1).

4-(2-methylsulfonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.27 (s, 3H), 2.92 (s, 3H), 3.39-3.53 (m, 2H), 3.71-3.88 (m, 2H), 5.31-5.39 (m, 1H), 6.17-6.33 (m, 1H), 7.36-7.43 (m, 3H), 8.20-8.25 (m, 2H), 9.52 (s, 1H); MS (ES): 360.2 (M^{+}+1).

Example 6:

A mixture of 4-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d] pyrimidine (0.70 g, 2.72 mmol) and 1,2-diaminoethane (10.0 mL, 150 mmol) was refluxed under inert atmosphere for 6 hr. The excess amine was removed *in vacuo*, the residue was washed sequentially with ether and hexane to give 0.75 g (98%) of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES); 282.2 (M^{+}+1), 265.1 (M^{+}-NH_3).

Example 7:

To a solution of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-

7*H*-pyrrolo[2,3d]pyrimidine (70.0 mg, 0.249 mmol) and triethylamine (50.4 mg, 0.498 mmol) in dichloromethane (2.0 mL) was added propionyl chloride (25.6 mg, 0.024 mL, 0.274 mmol) at 0°C. After 1 hr, the mixture was concentrated *in vacuo* and the residue was subjected to preparative thin layer chromatography (EtOAc) to give 22.0 mg (26%) of 4-(2-propionylaminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 338.2 (M^{+}+1).

The following compounds were obtained in a similar manner as that of Example 7:

4-(2-N'-methylureaethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.32 (s, 3H), 3.53 (d, 3H), 3.55 (m, 2H), 3.88 (m, 2H), 4.29 (m, 1H), 5.68 (t, 1H), 5.84 (m, 1H), 7.42 (m, 3H), 8.36 (dd, 2H), 9.52 (s, 1H); MS (ES): 339.3 (M^{+}+1).

4-(2-N'-ethylureaethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 353.2 (M^{+}+1).

Example 8:

To a solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41.1 mg, 0.215 mmol), dimethylaminopyridine (2.4 mg, 0.020 mmol) and pyruvic acid (18.9 mg, 0.015 mL, 0.215 mmol) in dichloromethane (2.0 mL) was added 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine (55.0 mg, 0.196 mmol). The mixture was stirred at room temperature for 4 hr. Usual workup and column chromatography (EtOAc) then gave 10.0 mg (15%) of 4-(2'-pyruvylamidoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine.. MS (ES): 352.2 (M^{+}+1).

Example 9:

To a solution of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (60.0 mg, 0.213 mmol) in dichloromethane (2.0 mL) was added N-trimethylsilyl isocyanate (43.3 mg, 0.051 mL, 0.320 mmol). The mixture was stirred at room temperature for 3 hr followed by addition of aqueous sodium bicarbonate. After filtration through small amount of silica gel, the filtrate was concentrated *in vacuo* to dryness to give 9.8 mg (14%) of 4-(2-ureaethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 325.2 (M^{+}+1).

The following compounds were obtained in a similar manner as that of Example 9:

dl-4-(2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28-1.32 (d, J=8 Hz, 3 H), 1.66 (s, 3H), 1.96 (s, 3H), 2.30 (s, 3H) 3.76-3.83 (m, 2H), 4.10-4.30 (m, 1H), 5.60-5.66 (t, J=6 Hz, 1H), 7.40-7.51(m, 3H), 8.36-8.43 (m, 2H), 10.83 (s, 1H); MS (ES): 338.2 (M^{+}+1).

(R)-4-(2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, 3H), 1.66 (s, 3H) 1.99 (s, 3H), 2.31 (s, 3H), 3.78-3.83 (m, 2H), 4.17-4.22 (m, 1H), 5.67 (t, 1H), 7.38-7.5 (m, 3H), 8.39 (m, 2H), 10.81 (s, 1H); MS (ES): 338.2 (M^{+}+1).

(R)-4-(1-methyl-2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (d, 3H), 1.68 (s, 3H), 2.21 (s, 3H), 2.34 (s, 3H), 3.46-3.52 (br, m, 2H), 4.73 (m, 1H), 5.22 (d, 1H), 7.41-7.46 (m, 3H), 8.36-8.40 (m, 2H), 8.93 (s, 1H); MS (ES): 338.2 (M^{+}+1).

(S)-4-(2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (d, 3H), 1.66 (s, 3H) 2.26 (s, 3H), 2.35 (s, 3H), 3.78-3.83 (m,

2H), 4.17-4.22 (m, 1H), 5.67 (t, 1H), 7.38-7.5 (m, 3H), 8.39 (m, 2H), 8.67(s, 1H); MS (ES): 338.2 (M^{+}+1).

(S)-4-(1-methyl-2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (d, 3H), 1.68 (s, 3H), 2.05 (s, 3H), 2.32 (s, 3H), 3.46-3.52 (m, 2H), 4.73 (m, 1H), 5.22 (d, 1H), 7.41-7.46 (m, 3H), 8.36-8.40 (m, 2H), 10.13 (s, 1H); MS (ES): 338.2 (M^{+}+1).

Example 10:

Reaction of 4-chloro-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d] pyrimidine with the mixture of *dl*-1-amino-2-(1,1-dimethyl ethoxy)carbonylamino-propane and *dl*-2-amino-1-(1,1-dimethyl ethoxy)carbonylamino-propane was run in a similar manner as that of Example 1. The reaction gave a mixture of *dl*-4-(1-methyl-2-(1,1-dimethylethoxy)carbonylamino)ethylamino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine and *dl*-4-(2-methyl-2-(1,1-dimethylethoxy)carbonylamino)ethylamino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine which were separated by column chromatography (EtOAc:hexanes=1:3). The first fraction was *dl*-4-(1-methyl-2-(1,1-dimethylethoxy) carbonylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29 - 1.38 (m, 12 H), 1.95 (s, 3H), 2.31 (s, 3H) 3.34-3.43 (m, 2H), 4.62-4.70 (m, 1H), 5.36-5.40 (d, J=8 Hz, 1H), 5.53 (br, 1H), 7.37-7.49(m, 3H), 8.37-8.44(m, 2H), 10.75 (s, 1H). MS 396.3 (M^{+}+1); The second fraction was *dl*-4-(2-(1,1-dimethylethoxy)carbonylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26-1.40 (m, 12 H), 2.00 (s, 3H), 2.31 (s, 3H) 3.60-3.90 (m, 2H), 3.95-4.10 (m, 1H), 5.41-5.44 (d, J=6.0 Hz, 1H), 5.65(br, 1H), 7.40-7.46(m, 3H), 8.37-8.44(m, 2H), 10.89 (s, 1H); MS (ES): 396.2 (M^{+}+1).

The following compounds were obtained in a similar manner as

that of Example 10:

(S,S)-4-(2-acetylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ_1.43 (m, 4 H), 1.60 (s, 3 H), 1.83 (m, 2 H), 2.18 (s, 3 H), 2.30 (m, 2 H), 2.32 (s, 3 H), 3.73 (br, 1H), 4.25 (br, 1H), 5.29 (d, 1H), 7.43-7.48 (m, 3H), 8.35-8.40 (m, 2H), 9.05 (s, 1 H).

4-(2-methyl-2-acetylaminopropyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ_1.51 (s, 6H), 1.56 (s, 3H), 2.07 (s, 3H), 2.36 (s, 3H), 3.76 (d, 2H), 5.78 (t, 1H), 7.41-7.48 (m, 3H), 7.93 (s, 1H), 8.39 (m, 2H), 10.07 (s, 1H); MS (ES): 352.3 (M^++1).

Example 11:

dl-4-(1-methyl-2-(1,1-dimethylethoxy) carbonyl aminoethyl) amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine (60.6 mg, 0.153 mmol) was treated with trifluoroacetic acid (0.5 mL) in dichloromethane (2.0 mL) for 14 hr. The organic solvent was removed *in vacuo* to dryness. The residue was dissolved in N,N-dimethylformamide (2.0 mL) and triethylamine (2.0 mL). To the solution at 0°C was added acetic anhydride (17.2 mg, 0.169 mmol). The resulted mixture was stirred at room temperature for 48 hr and then concentrated *in vacuo* to dryness. The residue was subjected to preparative thin layer chromatography (EtOAc) to give 27.0 mg (52%) of *dl*-4-(1-methyl-2-acetylaminoethyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^1H NMR (200 MHz, $CDCl_3$) δ 1.38-1.42 (d, J=8 Hz, 3 H), 1.69 (s, 3H), 2.01 (s, 3H), 2.32 (s, 3H) 3.38-3.60 (m, 2H), 4.65-4.80 (m, 1H), 5.23-5.26 (d, J=6 Hz, 1H), 7.40-7.51(m, 3H), 8.37-8.43(m, 2H), 10.44 (s, 1H); MS (ES): 338.2 (M^++1).

Example 12:

(R,R)-4-(2-aminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-

pyrrolo[2,3d]pyrimidine, prepared in a similar manner as that of Example 1 from 4-chloro-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (0.15 g, 0.583 mmol) and (1R, 2R)-(-)-1,2-diaminocyclohexane (0.63 g, 5.517 mmol), was treated with triethylamine (0.726 g, 7.175 mmol) and acetic anhydride (0.325 g, 3.18 mmol) in N,N-dimethylformamide (10.0 mL) at room temperature for 2 hr. After removal of solvent *in vacuo*, ethyl acetate (10.0 mL) and water (10.0 mL) were added to the residue. The mixture was separated and the aqueous layer was extracted with ethyl acetate (2 x 10.0 mL). The combined ethyl acetate solution was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo to dryness and the residue was subjected to column chromatography (EtOAc:Hexane=1:1) to give 57.0 mg (26%) of (R,R)-4-(2-acetylaminocyclohexyl)amino-5,6-dimethyl-2-phenyl-7H-pyrrolo[2,3d]pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.43 (m, 4 H), 1.60 (s, 3 H), 1.84 (m, 2 H), 2.22 (s, 3 H), 2.30 (m, 2 H), 2.33 (s, 3 H), 3.72 (br, 1H), 4.24 (br, 1H), 5.29 (d, 1H), 7.43-7.48 (m, 3H), 8.35-8.39 (m, 2H), 8.83 (s, 1 H); MS (ES): 378.3 (M^++1).

Example 13:

To a solution of 4-(2-hydroxyethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (40.0 mg, 0.141 mmol) in pyridine (1.0 mL) was added acetic anhydride (0.108 g, 1.06 mmol) at 0°C. The mixture was stirred at room temperature for 4 hr and the solvent was removed in vacuo. The residue was subjected to preparative thin layer chromatography (EtOAc:hexane=1:1) to give 32.3 mg (71%) of 4-(2-acetyloxyethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine. ^{1}H NMR (200 MHz, $CDCl_3$) δ_1.90 (s, 3H), 2.08 (s, 3H), 2.31 (s, 3H), 4.05 (m, 2H), 4.45 (t, 2H), 5.42 (m, 1H), 7.41-7.49 (m, 3H), 8.42(m, 2H), 11.23 (s, 1H).

Example 14:

A solution of Fmoc-β-Ala-OH (97.4 mg, 0.313 mmol) and oxalyl chloride (39.7 mg, 27.3 μL, 0.313 mmol) in dichloromethane (4.0 mL) with 1 drop of N,N-dimethylformamide was stirred at 0°C for 1 hr followed by addition of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (80.0 mg, 0.285 mmol) and triethylamine (57.6 mg, 79.4 μL, 0.570 mmol) at 0°C. After 3 hr, the mixture was concentrated *in vacuo* and the residue was treated with the solution of 20% piperidine in N,N-dimethylforamide (2.0 mL) for 0.5 hr. After removal of the solvent *in vacuo,* the residue was washed with diethyl ether:hexane (1:5) to give 3.0 mg (3%) of 4-(6-amino-3-aza-4-oxohexyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine. MS (ES): 353.2 (M^++1).

Example 15:

A solution of 4-(2-aminoethyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine (70.0 mg, 0.249 mmol) and succinic anhydride (27.0 mg, 0.274 mmol) in dichloromethane (4.0 mL) with 1 drop of N,N-dimethylformamide was stirred at room temperature for 4 hr. The reaction mixture was extracted with

20% sodium hydroxide (3 x 5.0 mL). The aqueous solution was acidified with 3 M hydrochloride to pH = 7.0. The whole mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic solution was dried ($MgSO_4$) and filtered. The filtrate was concentrated *in vacuo* to dryness to give 15.0 mg (16%) of 4-(7-hydroxy-3-aza-4,7-dioxoheptyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 382.2 (M^++1).

Example 16:

To 10 mL of dimethylformamide (DMF) at room temperature were added 700 mg of 4-(*cis*-3-hydroxycyclopentyl)amino-2-phenyl-5,6-dimethyl-7*H*-pyrrolo[2,3d]pyrimidine followed by 455 mg of N-Boc glycine, 20 mg of N,N-dimethylaminopyridine (DMAP), 293 mg of hydroxybenzotriazole (HOBT) and 622 mg of 1-(3-dimethylaminopropyl)-3-ethylcarboiimide hydrochloride (EDCl). The reaction mixture was left stirring overnight. DMF was then removed under reduced pressure and the reaction mixture was partitioned between 20mL of ethyl acetate and 50mL of water. The aqueous portion was extracted further with 2x20mL of ethyl acetate and the combined organic portions were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. Purification on silica gel, eluting with ethyl acetate/hexane gave 410 mg of the desired product: 4-(*cis*-3-(N-t-butoxycarbonyl-2-aminoacetoxy) cyclopentyl) amino-2-phenyl-5,6,-dimethyl-7*H*-pyrrolo[2,3d] pyrimidine, MS (ES) (M^++1)=480.2. The ester was then treated with 5 mL of 20% trifluoroacetic acid in dichloromethane at room temperature, left over night and then concentrated. Trituration with ethyl acetate gave 300 mg of an off white solid; 4-(*cis*-3-(2-aminoacetoxy)cyclopentyl)amino-5,6-dimethy 1-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine trifluoroacetic acid salt, MS (ES) (M^++1)=380.1.

One skilled in the art will appreciate that the following compounds can be synthesized by the methods disclosed above:

4-(*cis*-3-hydroxycyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine MS (ES) (M^{+}+1)= 323.1.

4-(*cis*-3-(2-aminoacetoxy)cyclopentyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidinetrifluoroacetic acid salt MS (ES) (M^{+}+1)= 380.1.

4-(3-acetamido)piperidinyl-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine
MS (ES) (M^{+}+1)= 364.2.

4-(2-N'-methylureapropyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine, MS (ES) (M^{+}+1)=353.4.

4-(2-acetamidobutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine,
MS (ES) (M^{+}+1)= 352.4.

4-(2-N'-methylureabutyl)amino-5,6-dimethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine
MS (ES) (M^{+}+1)= 367.5

4-(2-aminocyclopropylacetamidoethyl)amino-2-phenyl-7*H*-pyrrolo[2,3d] pyrimidine MS (ES) (M^{+}+1)= 309.1.

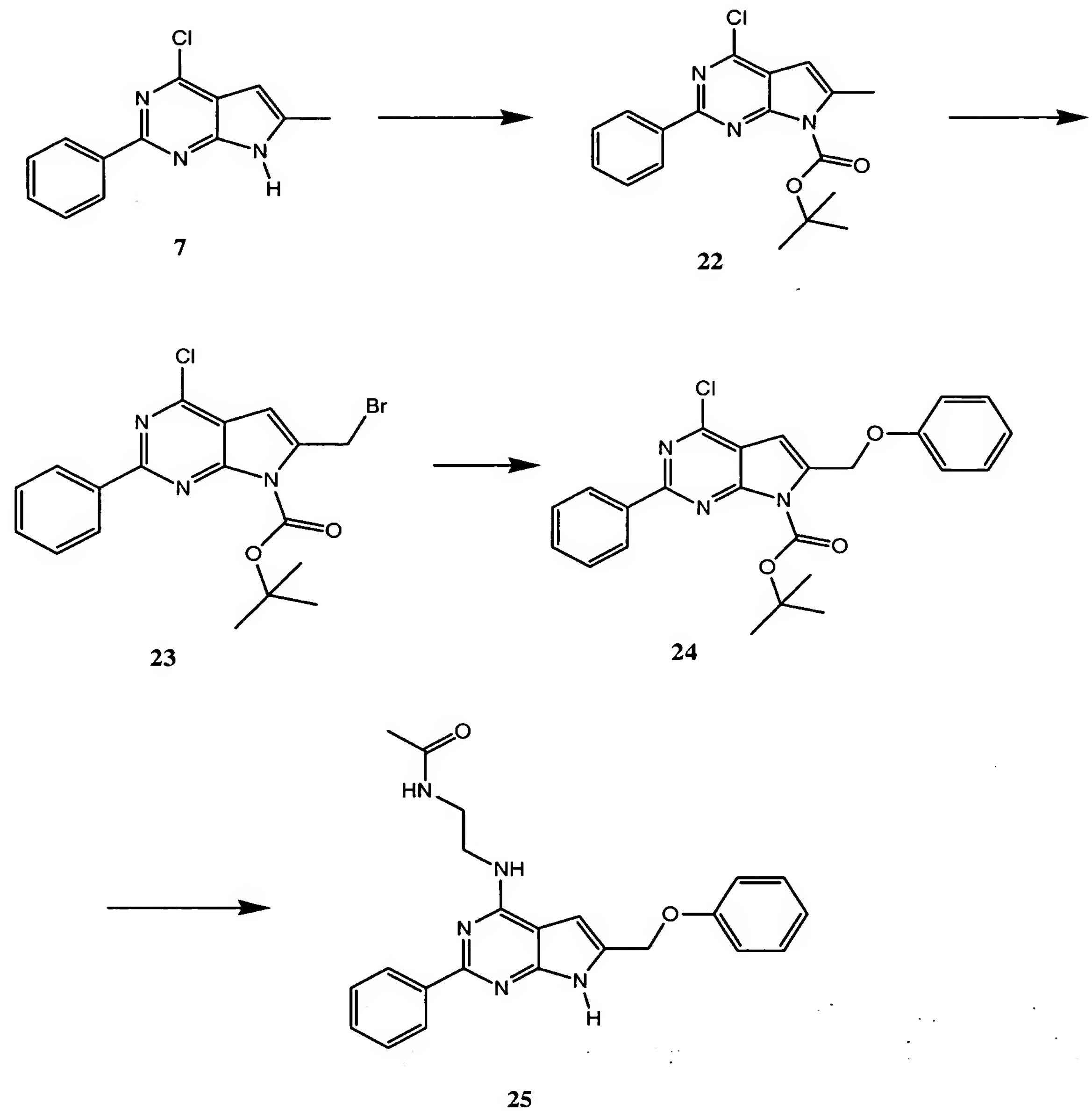
4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-chlorophenyl)-7*H*-pyrrolo[2,3d] pyrimidine MS (ES) (M^{+}+1)=342.8.

4-(*trans*-4-hydroxycyclohexyl)amino-2-(3-fluorophenyl)-7*H*-pyrrolo [2,3d] pyrimidine MS (ES) (M^{+}+1)=327.2.

4-(*trans*-4-hydroxycyclohexyl)amino-2-(4-pyridyl)-7*H*-pyrrolo[2,3d]pyrimidine MS (ES) (M^{+}+1)=310.2.

Example 17

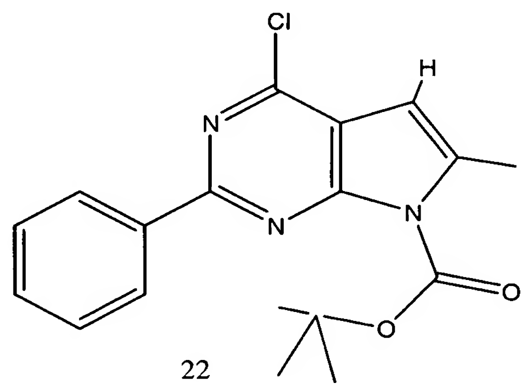
Scheme IX



The pyrrole nitrogen of (7) (Scheme IX) was protected with di-*t*-butyldicarbonate under basic conditions to yield the corresponding carbamate (22). Radical bromination of (22)

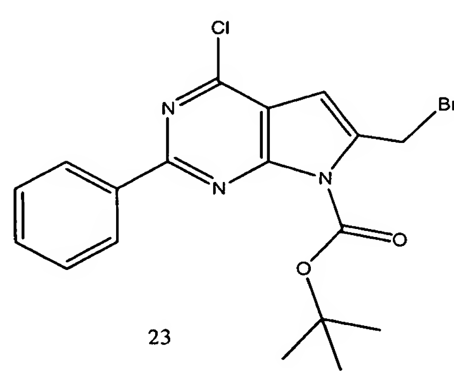
proceeded regioselectively to yield bromide (23). In general, compound (23) served as a key electrophilic intermediate for various nucleophilic coupling partners. Displacement of the alkyl bromide with sodium phenolate trihydrate yielded compound (24). Subsequent displacement of the aryl chloride and removal of the t-butyl carbamate protecting group occurred in one step yielding desired compound (25).

Detailed Synthesis of Compounds (22)-(25) in Accordance with Scheme IX



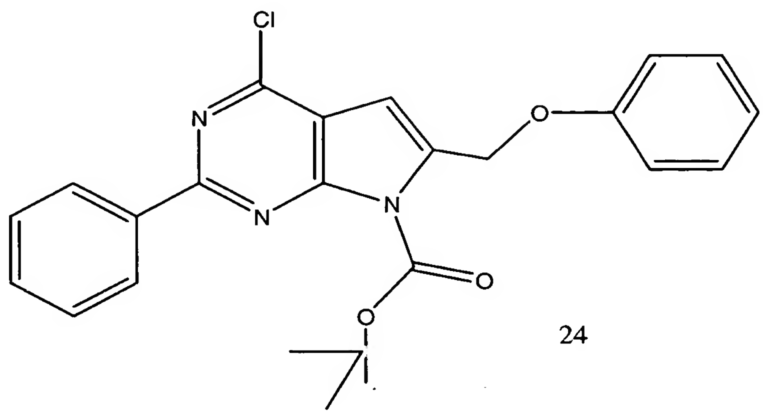
22

Di-*t*-butyl dicarbonate (5.37 g, 24.6 mmol) and dimethyl aminopyridine (1.13 g, 9.2 mmol) were added to a solution containing (7) (1.50 g, 6.15 mmol) and pyridine (30 mL). After 20 h the reaction was concentrated and the residue was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was separated, dried over $MgSO_4$, filtered and concentrated to yield a black solid. Flash chromatography (SiO_2; 1/9 EtOAc/Hexanes, R_f 0.40) yielded 1.70 g (80%) of a white solid (22). ^1H NMR (200 MHz, $CDCl_3$) δ_8.50 (m, 2H, Ar-H), 7.45 (m, 3H, Ar-H), 6.39 (s, 1H, pyrrole-H), 2.66 (s, 3H, pyrrole-CH_3), 1.76 (s, 9H, carbamate-CH_3); MS, M + 1 = 344.1; Mpt = 175-177°C.



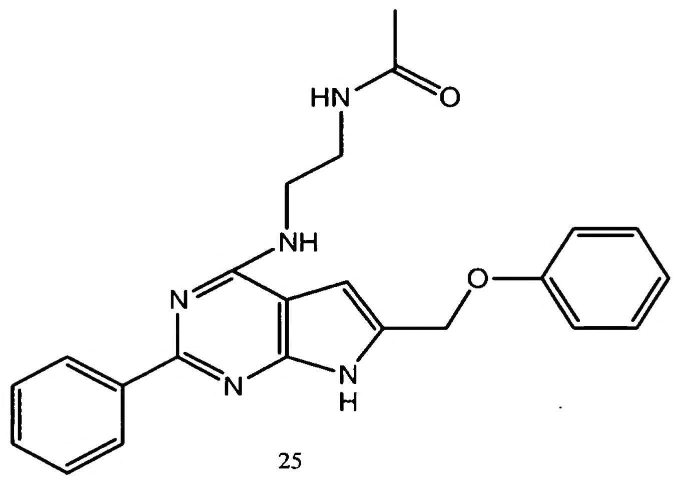
23

N-Bromosuccinimide (508 mg, 2.86 mmol) and AIBN (112 mg, 0.68 mmol) were added to a solution containing (22) (935 mg, 2.71 mmol) and CCl_4 (50 mL). The solution was heated to reflux. After 2 h the reaction was cooled to room temperature and concentrated *in vacuo* to yield a white solid. Flash chromatography (SiO_2; 1/1 CH_2Cl_2/Hexanes, R_f 0.30) yielded 960 mg (84%)of a white solid (23). ^1H NMR (200 MHz, $CDCl_3$) δ_8.52 (m, 2H, Ar-H), 7.48 (m, 3H, Ar-H), 6.76 (s, 1H, pyrrole-H), 4.93 (s, 2H,pyrrole-CH_2Br), 1.79 (s, 9H, carbamate-CH_3); MS, M + 1 = 423.9; Mpt = 155-157°C.



24

Sodium phenoxide trihydrate (173 mg, 1.02 mmol) was added in one portion to a solution of bromide (23) (410 mg, 0.97 mmol) dissolved in CH_2Cl_2 (5 mL) and DMF (10 mL). After 2 h the reaction solution was partitioned between CH_2Cl_2 and water. The water layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 layers were washed with water, dried over $MgSO_4$, filtered and concentrated to yield a yellow solid. Flash chromatography (SiO_2; 1/6 EtOAc/Hexanes, R_f 0.30) yielded 210 mg (50%) of a white solid (24). ^1H NMR (200 MHz, $CDCl_3$) δ_8.53 (m, 2H, Ar-H), 7.48 (m, 3H, Ar-H), 7.34 (m, 2H, Ar-H), 7.03 (m, 3H, Ar-H), 6.83 (s, 1H, pyrrole-H), 5.45 (s, 2H, $ArCH_2O$), 1.76 (s, 9H, carbamate-CH_3); MS, M^+ = 436.2.



25

A solution containing (24) (85 mg, 0.20 mmol), N-acetylethylenediamine (201 mg, 1.95 mmol) and DMSO (3 mL) was heated to 100°C. After 1 h the temperature was raised to 130°C. After 3 h the reaction was cooled to room temperature and partitioned between EtOAc and water. The water layer was extracted with EtOAc (2x). The combined EtOAc layers are washed with water, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (SiO_2; 1/10 EtOH/ $CHCl_3$, R_f 0.25) yielded 73 mg (93%)of a white foamy solid (25). ^1H NMR (200 MHz, DMSO-$_{d6}$) δ 11.81 (br s, 1H, N-H), 8.39 (m, 2H, Ar-H), 8.03 (br t, 1H, N-H), 7.57 (br t, 1H, N-H), 7.20 - 7.50 (m, 5H, Ar-H), 6.89 - 7.09 (m, 3H, Ar-H), 6.59 (s, 1H, pyrrole-H), 5.12 (s, 2H, Ar*CH₂*O), 3.61 (m, 2H, N*CH₂*), 3.36 (m, 2H, N*CH₂*), 1.79 (s, 3H, CO*CH₃*); MS, M+ 1 = 402.6

The following compounds were obtained in a manner similar to that of Example 17:

4-(2-acetylaminoethyl)amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. mp 196-197°C; MS (ES): 401.6 (M^++1).

4-(2-acetylaminoethyl)amino-6-(4-fluorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 420.1 (M^++1).

4-(2-acetylaminoethyl)amino-6-(4-chlorophenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 436.1 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(4-methoxyphenoxy)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 432.1 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(N-pyridin-2-one)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 403.1 (M^{+}+1).

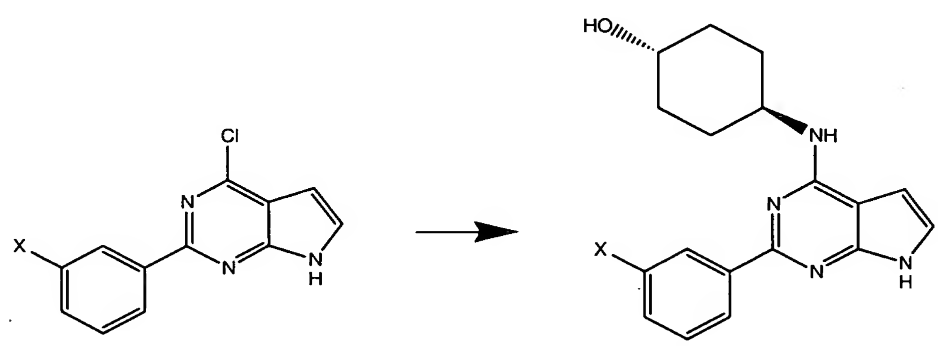
4-(2-acetylaminoethyl)amino-6-(N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3]pyrimidine. MS(ES): 400.9 (M^{+}+1).

4-(2-acetylaminoethyl)amino-6-(N-methyl-N-phenylamino)methyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS(ES): 414.8 (M^{+}+1).

4-(2-N'-methylureaethyl)amino-6-phenoxymethyl-2-phenyl-7*H*-pyrrolo[2,3d]pyrimidine. MS (ES): 416.9 (M^{+}+1).

Example 18: Synthesis of adenosine A_1 Antagonists.

Compound 1319 and Compound 1320 (Table 13 below) can be synthesized by the general procedures given below.



Compound 26 X=F
Compound 27 X=Cl

Compound 1319
Compound 1320

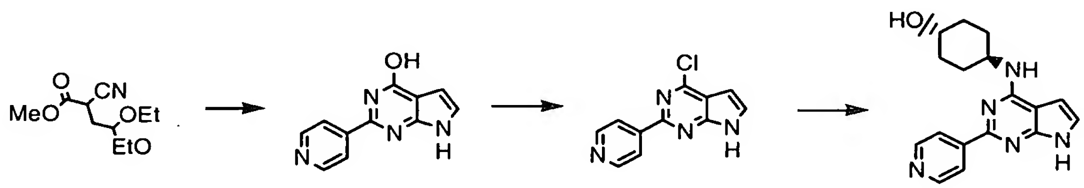
Compound 1319 (81%) ^{1}H-NMR (d_6-DMSO) d 1.37 (m, 4H), 1.93 (m, 2H), 2.01 (m, 2H), 4.11 (brs, 1H), 4.61 (d, 1H, J = 4.4 Hz), 6.59 (m, 1H), 7.09 (m, 1H), 7.21 (m, 2H), 7.49 (dd, 1H, J = 8Hz, 14Hz), 8.03 (m, 1H), 8.18 (d, 1H, J = 8 Hz), 11.55 (brs,

1H). MS (ES): 327.0 (M^{+}+1).

Compound 1320 (31%) MS (ES): 343.1 (M^{+}+1).

Example 19: Synthesis of adenosine A_1 Antagonist.

Compound 1321 (Table 13 below) can be synthesized by the general procedures given below.



28 29 30 Compound 1321

Compound 28 (10.93g, 50.76 mmol) was dissolved in DMF (67 mL). 4-Amidinopyridine hydrochloride (8.0g, 50.76 mmol) and DBU (15.4 g, 101.5 mmol) were added sequentially and the reaction was heated to 85°C. After 22 hours, the reaction was cooled to room temperature and the DMF was removed in vacuo. The dark oil was diluted with 2M HCl (80 mL). The reaction was allowed to stand. After 2 hours, the solution was cooled to 10°C and filtered. The solid was washed with cold water and dried to yield 7.40g of a yellow solid, Compound 29 (69%). ^{1}H-NMR (200MHz, d_6-DMSO) d 6.58 (s, 1H), 7.27 (s, 1H), 8.53 (d, 2H, J = 5.6), 9.00 (d, 2H, J = 5.2Hz), 12.35 (brs, 1H). MS (ES): 212.8 (M^{+}+1).

Compound 29 (7.4 g, 29.8 mmol) was diluted with $POCl_3$ and heated to 105°C. After 18 hours, the reaction is cooled to room temperature and the $POCl_3$ is removed in vacuo. The thick dark oil is diluted with MeOH (75mL) followed by ether (120mL). The amorphous red solid is filtered and washed with ether to yield 3.82 g of a red solid. The crude solid is approximately 80% pure and used without further purification in the next reaction. MS (ES): 230.7 (M^{+}+1).

Compound 1321 ^{1}H-NMR (15%) (200MH, d_6-DMSO) d 1.38 (m, 4H), 1.92 (brs, 2H), 2.02 (brs, 2H), 3.44 (brs, 1H), 4.14 (brs, 1H), 4.56 (d, 1H, J = 4 Hz), 6.63 (m, 1H), 7.15 (m, 1H), 7.32 (d, 1H, J = 6.2 Hz), 8.20 (d, 2H, J = 4.4 Hz), 8.65 (d, 2H, J = 4.4Hz), 11.67 (brs, 1H). MS (ES): 310.2 (M^++1).

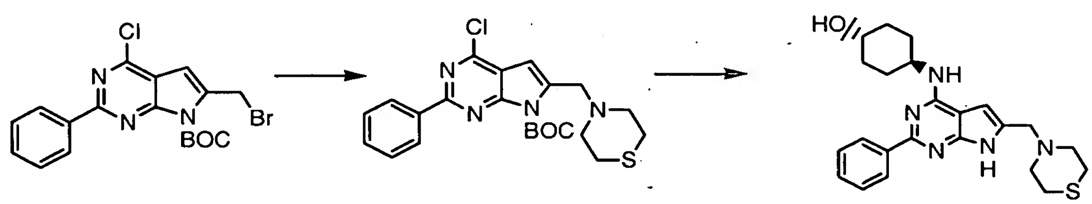
Compound 1501 (Table 15 below) ^{1}H-NMR (70%) (200MHz, CD_3OD) d 1.84 (s, 3H), 3.52 (t, 2H, J = 6.0 Hz), 3.83, t, 2H, J = 6.0 Hz), 6.51 (d, 1H, J = 3.4Hz), 7.06 (d, 1H, J = 3.8 Hz), 7.42 (m, 3H), 8.36 (m, 2H). MS (ES): 296.0 (M^++1).

Compound 1502 (Table 15 below) MS (ES): 345.0 (M^++1).

Compound 1500 (Table 15 below) ^{1}H-NMR (200MHz, $CDCl_3$) d 1.40 - 1.80 (m, 6H), 1.85 - 2.10 (m, 2H), 2.18 (s, 3H), 2.33 (s, 3H), 2.50 (d, 3H), 3.90 - 4.10 (m, 2H), 4.76 (m, 1H), 5.50 (d, 1H), 6.03 (m, 1H), 7.40 (m, 3H), 8.37 (m, 2H), 9.15 (brs, 1H). MS (ES): 393.3 (M^++1).

Example 20A: Synthesis of adenosine A_1 Antagonist.

Compound 1504 (Table 15 below) can be synthesized by the general procedures given below.



31 32 Compound 1504

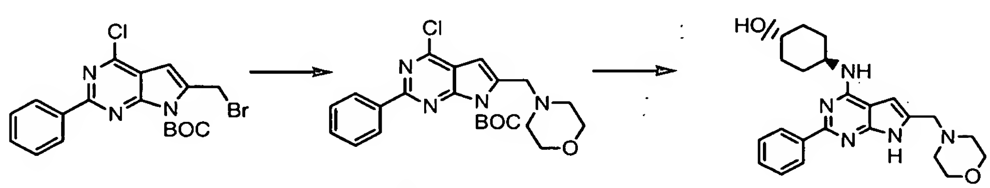
Compound 31 (200 mg, 0.47 mmol) was dissolved in DCM (4 mL). Triethylamine (51 mg, 0.5mmol) and thiomorpholine (52 mg, 0.5mmol) were added sequentially. The solution was mixed for several minutes and allowed to stand for 72 hours. The reaction was diluted with DCM and H_2O and the layers were

separated. The aqueous layer was extracted with DCM. The combined DCM layers were dried over $MgSO_4$, filtered and concentrated. Ethyl ether was added to the crude sample and the resulting solid was filtered to yield 100mg of a white solid, 32(62%). ^{1}HNMR (200MHz, $CDCl_3$) d 1.76 (s, 9H), 2.66 (brs, 2H), 2.79 (brs, 2H), 3.86 (s, 2H), 7.46 (m, 3H), 8.50 (m, 2H).

Compound 32 was combined with DMSO (3mL) and trans-4-aminocyclohexanol (144mg, 1.25 mmol) and heated to 130°C for 4 hours. The reaction was cooled to room temperature, and diluted with EtOAc and H_2O. The layers were separated and the aqueous layer was extracted with EtOAc (2x). The combined organic layers were washed with H_2O and brine, dried over MgSO4, filtered and concentrated. Chromatography (silica, 8:1 CHCl3/EtOH) yields 32 mg of a tan oil. Ethyl ether was added and the resulting solid was filtered to yield 5 mg of a white solid (9%).OSIC-148265: ^{1}H-NMR (200MHz, CD_3OD): d 1.44 (brm, 4H), 2.03 (brm, 2H), 2.21 (brm, 2H), 2.70 (brm, 8H), 3.63 (m, 4H), 3.92 (m, 1H), 4.26 (brs, 1H), 6.42 (s, 1H), 7.42 (m, 3H), 8.33 (m, 2H).

Example 20B: Synthesis of adenosine A_1 Antagonist.

Compound 1503 (Table 15 below) can be synthesized by the general procedures given below.



31 33 Compound 1503

The bromide, compound 31 (220 mg, 0.47 mmol) was dissolved in 1:1 DMF:Dichloromethane (5 mL). To this was added K_2CO_3 (71 mg,

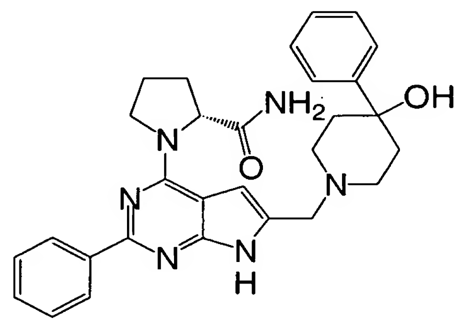
0.52 mmol) and morpholine (0.047 mL, 0.47 mmol). The mixture was allowed to stir at room temperature overnight. Solvents were removed in vacuo and the residue was partitioned between H_2O and dichloromethane. The organic layer was dried with $MgSO_4$, filtered, and concentrated to give an off white solid which upon trituration with ether/hexanes gave 175mg of a white solid, 33 (84%). ^{1}H-NMR (200MHz, $CDCl_3$): (1.9 (9H, s), 2.54 (4H, s), 3.65 (4H, s), 3.85 (1H, s), 6.59 (1H, s), 7.45 (3H, m),8.5 (2H, m).

Compound 33 (50 mg, 0.11 mmol) and trans-4-aminocyclohexanol (105 mg, 0.91 mmol) were taken up in DMSO (2mL). The resultant solution was sparged with N_2 and then heated to 100^{0}C in an oil bath and stirred overnight. The crude reaction mixture was poured into water and extracted twice with ethyl acetate (50mL). The combined organic layers were washed with H_2O. After drying with $MgSO_($ and filtering, the organic layer was concentrated in vacuo to give an orange solid. Chromatography (silica, 10% CH_3OH in CH_2Cl_2) yielded 15mg (33%). ^{1}H-NMR (200 MHz, $CDCl_3$): (1.24 - 1.62 (4H, m), 1.85 (2H, m), 2.10 (2H, m), 2.26 (4H, m), 3.53 (4H, m), 4.22 (1H, m), 4.73 (1H, m), 5.85 (1H, d), 6.15 (1H, s), 7.25 (3H, m), 8.42 (2H, M), 10.0 (1H, s). MS (ES): 408 (M^+ + 1).

Compounds 1500, 1501, and 1502 can be synthesized using similar preparation steps of Example 20 by treating compound 32 with an appropriately substituted amine.

Example 21: Synthesis of 1-[6-(4-Hydroxy-4-phenyl-piperidin-1-yl-methyl)-2-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pyrrolidine-2-carboxylic acid amide (1601).

Compound **1601** was synthesized in a manner similar to that of Example 17 using synthesis scheme IX with L-prolineamide and 4-phenyl-piperidin-4-ol to obtain:

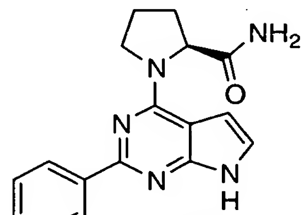


1601

^{1}H-NMR (d_6-DMSO) d 1.53 (s, 1H), 1.60 (s, 1H), 1.84-2.30 (m, 6H), 2.66 (m, 2H), 3.60 (s, 2H), 3.88 (m, 1H), 4.02 (m, 1H), 4.66 (d, 1H, J = 6.8Hz), 4.73 (s, 1H), 6.44 (s, 1H), 6.94 (s, 1H), 7.12 - 7.50 (m, 10H), 8.35 (m, 2H), 11.6 (brs, 1H); MS (ES): 305.1 (M^++1); mp = 234-235°C.

Example 22: Synthesis of [N-(2-Phenyl-7H-pyrrolo [2,3-d]pyrimidin-4-yl)(L)-prolinamide (1602)

Compound **1602** was synthesized using synthesis scheme VII with L-prolineamide to obtain:

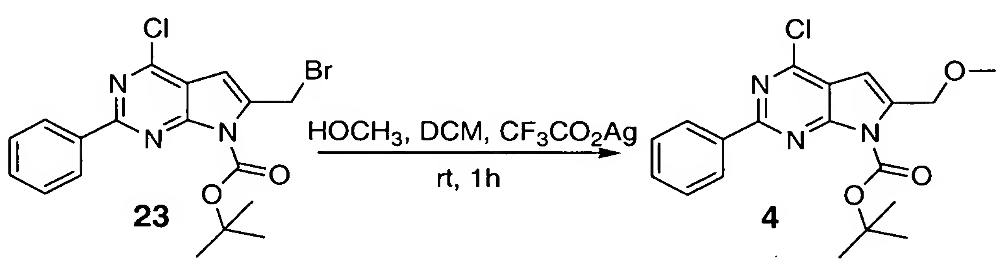


1602

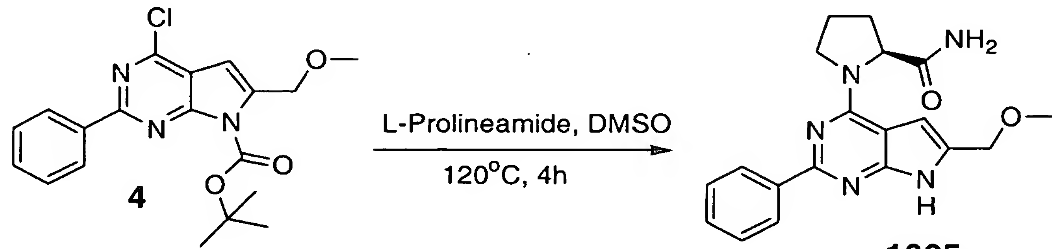
^{1}H-NMR (DMSO-d_6) d 2.05 (m, 4H), 3.85 (m, 1H), 4.05 (m, 1H), 4.70 (d, 1H, J=8.0Hz), 6.58 (brs, 1H), 6.95 (brs, 1H), 7.15 (d, 1H, J=3.4Hz), 7.40 (m, 3H), 7.50 (brs, 1H), 8.40 (m, 2H), 11.6 (brs, 1H); MS (ES): 308.3 (M^++1). mp= 236-238°C.

Example 23: Synthesis of [*N*-(2-phenyl-6-methoxymethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-(L)-prolinamide (1605)

Compound **1605** was synthesized using precursor compound 23 of synthesis scheme IX to obtain:



Bromide **23** (4.23g, 10mmol) is dissolved in anhydrous methanol (60mL) and DCM (120mL) and treated with AgO_2CCF_3 under N_2 at rt for 1h. The solid is removed by filtration and washed with DCM (2x20mL). The filtrate is concentrated *in vacuo*. The residue is redissolved in DCM (80mL). The resulted solution is then washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated to give 3.71g (**4**, 99%) off white solid. ^{1}H-NMR ($CDCl_3$) d 1.75 (s, 9H), 3.51 (s, 3H), 4.83 (s, 2H), 6.70 (s, 1H), 7.47 (m, 3H), 8.52 (m, 2H).

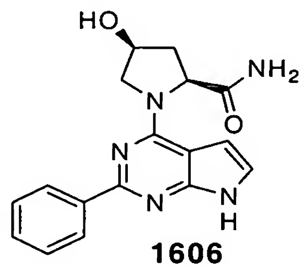


1605

Aryl chloride **4** (2.448g, 6.55mmol), DMSO (15mL), L-prolineamide (4.0g, 35.0mmol) and $NaHCO_3$ (2.9g) are combined and heated to 120°C under nitrogen. After 4h, the reaction is cooled to room temperature and diluted with water (60ml). The resulted slurry is extracted with DCM (10x). The combined organic layers are washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated to give 2.48g brown solid. Pure product (1.86g, 81%) is obtained after flash column as white solid. White crystals are gotten from THF/hexane. M.p. = 213-215°C. ^{1}H-NMR ($CDCl_3$) d 2.15 (m, 3H), 2.52 (m, 1H), 3.26 (s, 3H), 3.92 (m, 1H), 4.10 (m, 1H), 4.42 (s, 2H), 5.08 (d, 1H, J=8.2Hz), 5.49 (brs, 1H), 6.48 (s, 1H), 7.08 (brs, 1H), 7.42 (m, 3H), 8.38 (m, 2H), 9.78 (brs, 1H); MS (ES): 352.2 (M^++1).

Example 24: Synthesis of 4-Hydroxy-1-(2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-pyrrolidine-2-carboxylic acid amide (1606)

Compound **1606** was obtained with synthesis scheme VII using *cis*-hydroxy prolineamide to obtain:

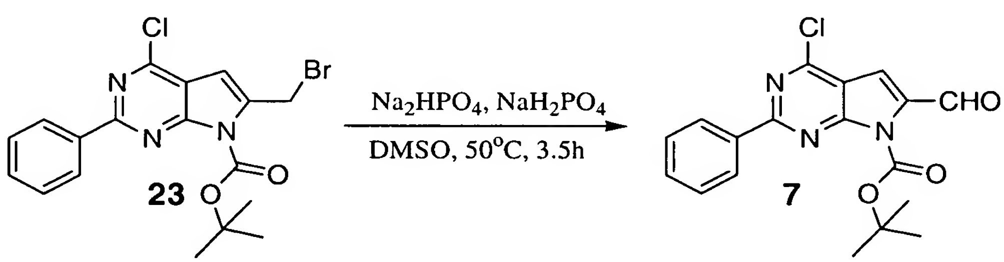


1606

^{1}H-NMR (d_6-DMSO) d 1.90 (m, 1H), 3.85 (d, 1H, J = 9.2Hz), 4.08 (m, 1H), 4.37 (s, 1H), 4.67 (dd, 1H, J = 8.8, 4.0Hz), 5.30 (s, 1H), 6.55 (s, 1H), 7.15 (s, 2H), 7.37 (m, 3H), 7.64 (s, 1H), 8.37 (m, 2H), 11.65 (brs, 1H); MS (ES): 324.2 (M^{+}+1); mp = 268-271°C.

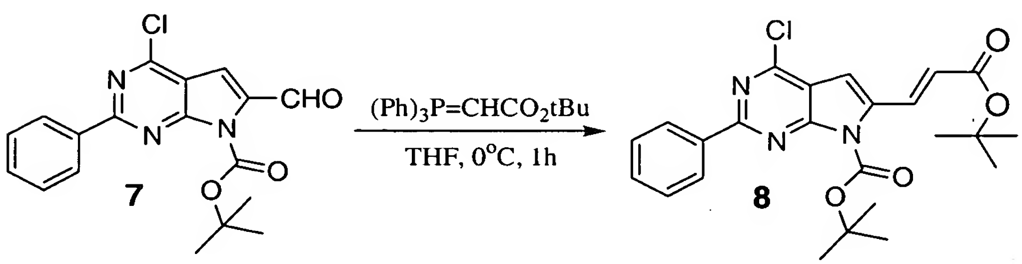
Example 25: Synthesis of 3-[4-((S)-2-Carbamoyl-pyrrolodin-1-yl)-2-phenyl-7*H*-pyrrolo[2,3-d]pyrimidin-6-yl]-propionic acid (1611)

Compound **1611** was obtained using precursor compound 23 of synthesis scheme IX to obtain:

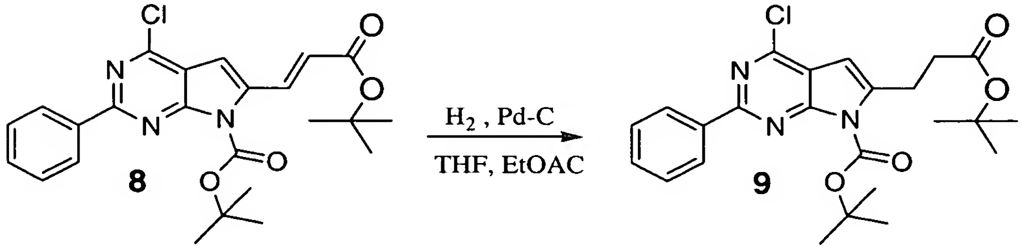


The tert-butoxycarbonyl protected aryl bromide **23** (4.0g, 9.5mmol), dry DMSO (25ml), NaH_2PO_4 (454mg, 3.79mmol) and Na_2HPO_4 (1.62g, 11.4mmol) were combined and heated to 50°C under argon for approximately 3.5h. The mixture was then

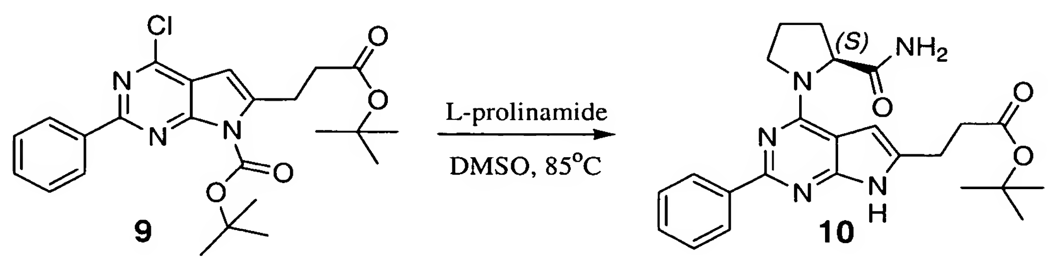
poured into water (200ml) and extracted with three 100ml portions of EtOAc. The combined organic layers were thoroughly washed with water, brine, dried over $MgSO_4$, filtered and concentrated to give a yellow solid which was purified by triturating with ethanol. to give 1.55g of a pale yellow solid (**7**). The mother liquor was purified by flash chromatography (10% EtOAc in hexane) to give an additional 454mg (60%). ^{1}H-NMR ($CDCl_3$) d 1.77 (s, 9H), 7.25 (s, 1H), 7.48 (m, 3H), 8.52 (m, 2H) 10.39 (s, 1H); m.p.= 156°C (dec).



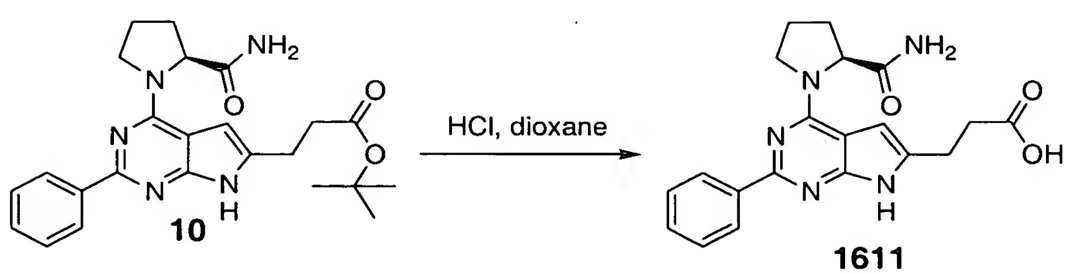
Aldehyde **7** (600mg, 1.7mmol) was dissolved in dry THF (20ml) and cooled to 0°C under argon. To this was added a 0°C solution of (tert-butoxycarbonylmethylene)-triphenylphosphorane (694mg, 1.8mmol) in 10ml of dry THF dropwise through a cannula. After 3h the mixture was concentrated and purified by triturating with ethanol to give 565mg (73%) of a white solid (**8**). ^{1}HNMR ($CDCl_3$) d 1.58 (s, 9H), 1.79 (s, 9H), 6.46 (d, 1H), 6.95 (s, 1H), 7.48 (m, 3H), 8.09 (d, 1H), 8.56 (m, 2H).



A solution of compound **8** (565mg 1.2mmol) in 5ml THF was diluted to 100ml with EtOAc. After adding 600mg of catalyst (5% wt Pd, 50% H_2O) and purging with argon, the mixture was hydrogenated under atmospheric pressure. After 8h the mixture was filtered, concentrated and purified with flash chromatography (10% EtOAc in hexane) to isolate 200mg (35%) of **9** as a clear oil that crystallized upon standing. ^{1}HNMR ($CDCl_3$) d 1.42 (s, 9H), 1.75 (s, 9H), 2.65 (t, 2H), 3.32 (t, 2H), 6.41 (s, 1H) 7.45 (m, 3H), 8.51 (m, 2H).



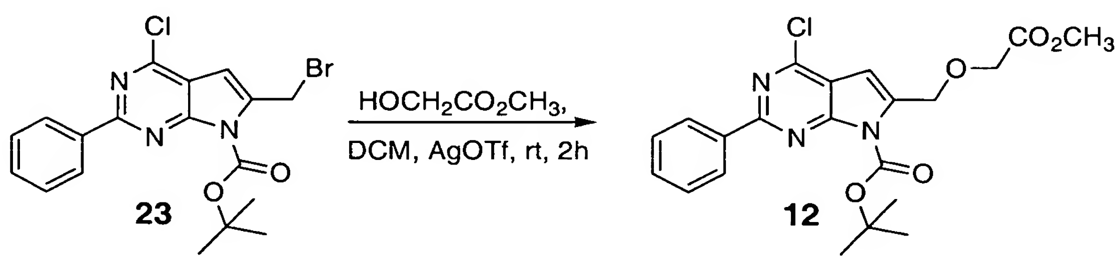
Aryl chloride **9** (200mg, 0.44mmol), DMSO (10ml) and L-prolinamide (440mg, 4.4mmol) were combined and heated to 85°C under argon. After 14 hours the mixture is cooled to room temperature and partitioned between water and ethyl acetate. The layers were separated and the aqueous layer washed with EtOAc (3x). The combined organic layers were thoroughly washed with water (3x), brine, dried over $MgSO_4$, filtered and concentrated to give **10** as a yellow film which was purified by flash chromatography (2.5% MeOH in CH_2Cl_2). 185mg (97%). MS (ES): 435.8 (M^++1).



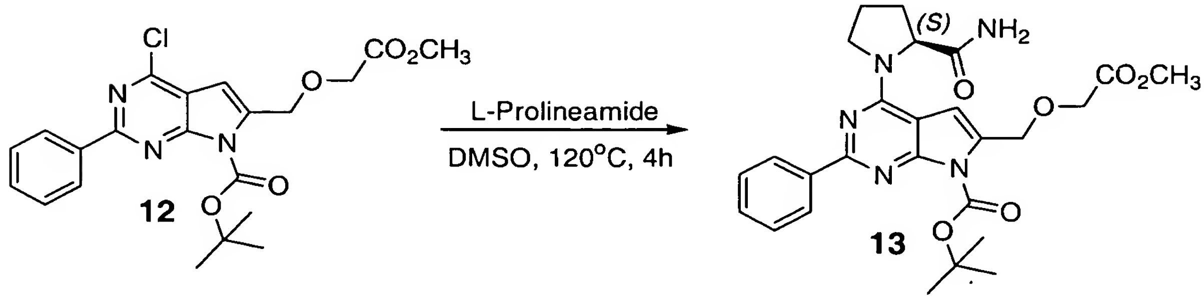
Ester **10** (30mg, mmol) in 5ml dioxane was hydrolyzed by adding 0.5ml concentrated HCl. After 3 hours the mixture was concentrated in vacuo and recrystalized in EtOH/ EtOAc to obtain **1611** as a white solid (20mg, 61%). MS (ES): 380 (M^{+}+1).

Example 26: Synthesis of [*N*-(2-phenyl-6-aminocarbonyl methoxymethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-(L)-prolinamide (1614)

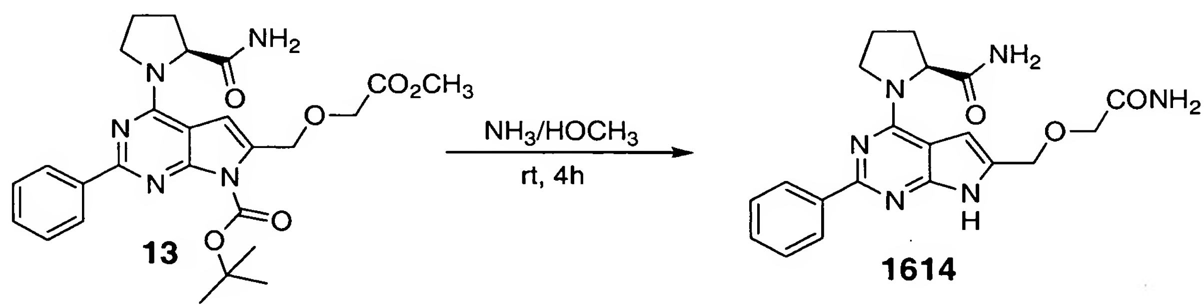
Compound **1614** was obtained using precursor compound 23 of synthesis scheme IX to obtain:



Bromide **23** (1.27g, 3mmol) and molecular sieve (5g) are stirred in anhydrous methyl glycolate (5.8g, 60mmol) and DCM (40mL). The solution is treated with AgOTf under N_2 and allowed to stir for 3h. The solid is removed by filtration and washed with DCM (2x20mL). The filtrate is concentrated *in vacuo.* The residue is redissolved in DCM (80mL). The resulted solution is then washed with water, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated to give 1.35g (99%) off white solid (**12**). ^{1}H-NMR ($CDCl_3$) d 1.75 (s, 9H), 3.80 (s, 3H), 5.0 (s, 2H), 6.78 (s, 1H), 7.47 (m, 3H), 8.52 (m, 2H).



Aryl chloride **12** (177mg, 0.41mmol), DMSO (10mL), L-prolinamide (466mg, 4mmol) and $NaHCO_3$ (500mg) are combined and heated to 120°C under nitrogen. After 4h, the reaction is cooled to room temperature and diluted with water (60ml). The resulted slurry is extracted with DCM (5x30mL). The combined organic layers are washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated to give brown solid. Pure product (154mg, 92%) is obtained after flash column as white solid (**13**). ^{1}H-NMR ($CDCl_3$) d 2.15 (m, 3H), 2.52 (m, 1H), 3.55 (s, 3H), 4.58 (s, 2H), 5.08 (s, 1H,), 5.85 (brs, 1H), 6.48 (s, 1H), 7.08 (brs, 1H), 7.42 (m, 3H), 8.40 (m, 2H), 10.58 (brs, 1H); MS (ES): 410.1 (M^++1).

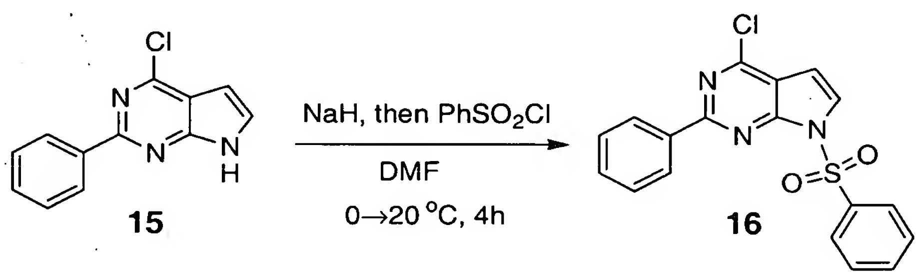


Methyl ester **13** (124mg, 0.3mmol) is dissolved in $HOCH_3$ (15mL). Ammonia is bubbled through the solution for 0.5h. The reaction mixture is then stirred for another 3h at rt. After removal

of solvent 111mg of a white solid (**1614**, 93%) is obtained. ^{1}H-NMR ($CDCl_3$) d 1.82 (m, 3H), 2.20 (m, 1H), 2.80 (m, 1H), 3.10 (m, 1H), 3.63 (dd, 2H, J_1=13.8Hz, J_2=19.4Hz), 3.87 (m, 1H), 4.07 (m, 1H), 4.97 (m, 1H), 5.96 (m, 2H), 6.35 (s, 1H), 6.86 (brs, 1H), 7.11 (brs, 1H), 7.37 (m, 3H), 8.28 (m, 2H), 11.46 (brs, 1H); MS (ES): 394.8 (M^++1).

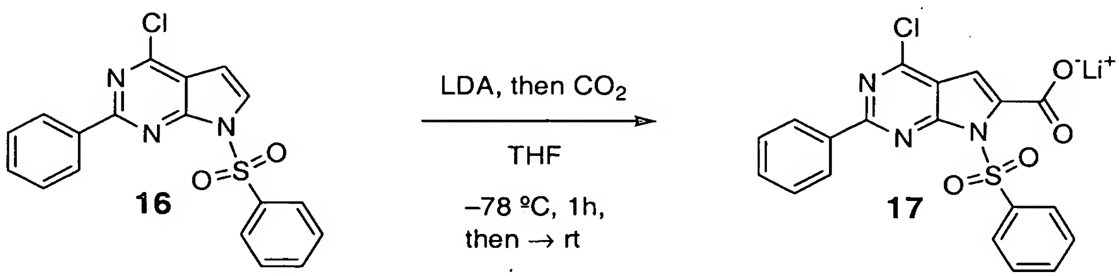
Example 27: Synthesis of [4-(2-Carbamoylpyrrolidin-1-yl)-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid] (1619)

Compound **1619** was synthesized using precursor compound **15** of synthesis scheme VII to obtain:

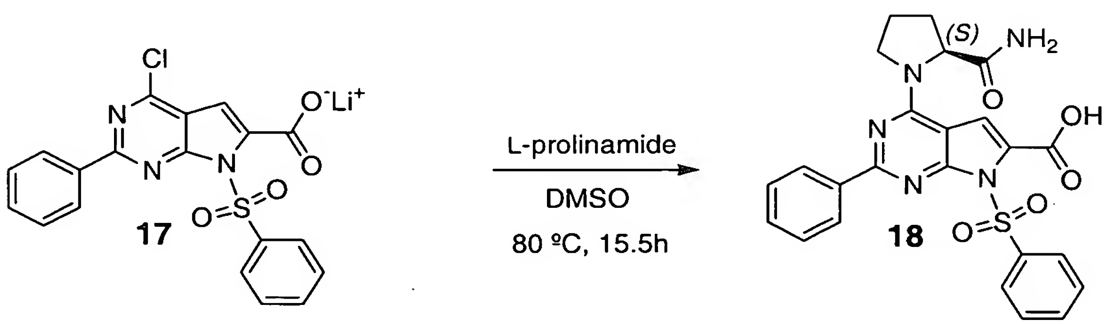


To a suspension of sodium hydride (780mg of a 60% oil suspension, 19.5mmol) in dry DMF (20mL), cooled by an ice/water bath, under nitrogen, is added a solution of the pyrrolopyrimidine **15** (2.00g, 7.52mmol) in DMF (10mL) over 5 min. After 15 min, benzenesulfonyl chloride (1.2mL, 9.40mmol) is added, then the cooling bath is removed. After 4h, the reaction mixture is poured into a mixture of ice and sat. $NaHCO_3$ sol., the precipitated solid is filtered off and triturated with acetone (3) and methanol (2), yielding 2.37g of a beige solid. This solid (**16**) contains approx. 10mol-% DMF (based on that 83% yield) and can be used in the next step; a pure sample can be obtained by chromatography on silica gel using acetone as eluent. ^{1}H-NMR ($CDCl_3$): d 6.70

(d, J = 4.2Hz, 1H), 7.47-7.68 (m, 6H), 7.76 (d, J = 4.2Hz, 1H), 8.24-8.32 (m, 2H), 8.48-8.56 (m, 2H); IR (solid): n = 3146 cm^{-1}, 1585, 1539, 1506, 1450, 1417, 1386, 1370, 1186, 1176, 1154, 1111, 1015, 919, 726, 683, 616, 607; MS (ES): 372/370 (MH^+); mp = 226-227 ºC.

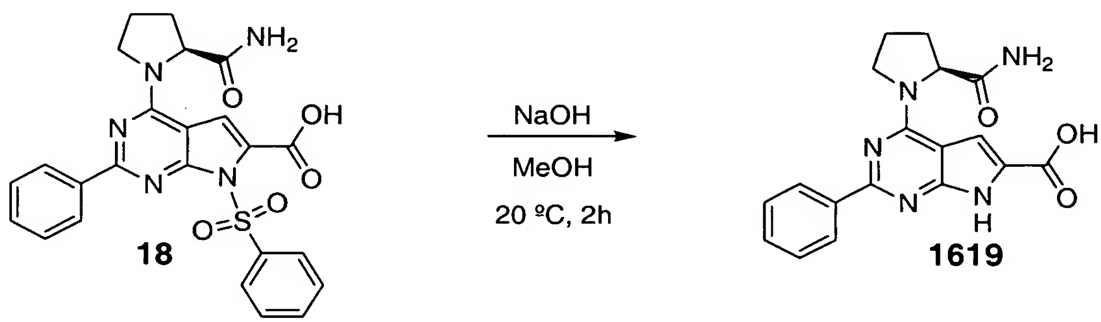


To a solution of the N-sulfonyl compound **16** (337mg, 0.911mmol) in dry THF (34mL), cooled by dry ice/acetone, is added LDA·THF (1.0mL, 1.5M solution in cyclohexane, 1.5mmol). After 45min, carbon dioxide is bubbled into the solution for 5min, then the cooling bath is removed. When the solution has reached ambient temp., the solvents are evaporated, yielding 398mg of the salt **17**, containing 0.5 equiv. of $(iPr)_2NCO_2Li$, as yellow solid. The salt is used without purification in the next step. ^{1}H-NMR (D_6-DMSO): d = 6.44 (s, 1H), 7.50-7.75 (m, 6H), 8.33-8.40 (m, 2H), 8.53 (dd, J = 8.0, 1.6Hz, 2H).



A solution of the lithium salt **17** (50mg) and L-prolinamide (122mg, 1.07mmol) in DMSO (1.5mL) is heated under nitrogen to

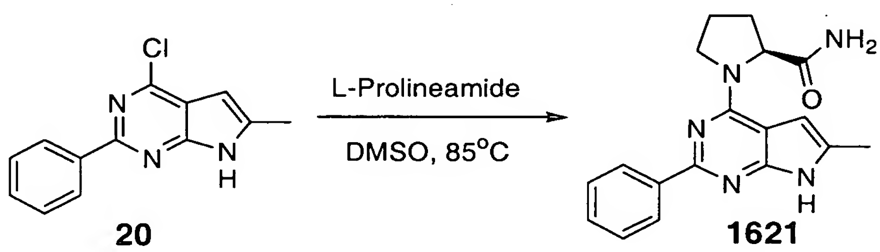
80 ºC for 15.5h. 4% aq. acetic acid (10mL) is added to the cooled solution, and the mixture is extracted with EtOAc (5´10mL). The combined organic layers are washed with 4% aq. acetic acid (10mL), water (10mL) and brine (10mL) and are dried over MgSO4. Filtration and concentration gives 40mg of **18** as a yellowish solid, which is used without purification in the next step. ^{1}H-NMR (CD_3OD): d = 1.95-2.36 (m, 4H), 3.85-3.95 (m, 1H), 3.95-4.17 (m, 1H), 4.72 (brs, 1H), 7.14 (s, 1H), 7.35-7.45 (m, 3H), 7.45-7.70 (m, 3H), 8.33-8.50 (m, 4H).



A solution of sodium hydroxide in methanol (1.5mL, 5M, 7.5mmol) is added to a solution of the pyrrolopyrimidine **18** (40mg, 0.081mmol) in methanol (2mL). After 2h, the pH is adjusted to 5, most of the methanol is evaporated, the mixture is extracted with EtOAc (5 10mL), the combined organic layers are washed with brine and dried over $MgSO_4$. Filtration and concentration yields 24mg of a pale yellow solid, which is triturated with toluene/EtOAc/MeOH to yield 15.6mg (55%) of the acid **1619** as slightly yellowish solid. ^{1}H-NMR (CD_3OD): d = 2.05-2.20 (m, 4H), 3.95-4.10 (m, 1H), 4.15-4.25 (m, 1H), 4.85 (brs, 1H), 7.14 (s, 1H), 7.35-7.42 (m, 3H), 8.38-8.45 (m, 2H); IR (solid): n = 3192 cm^{-1}, 2964, 2923, 2877, 1682, 1614, 1567, 1531, 1454, 1374, 1352, 1295, 1262, 1190, 974, 754, 700; MS (ES): 352 (M^++1); m.p. = 220 ºC (decomp.).

Example 28: Synthesis of 1-(6-methyl-2-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-4-yl)-(S)-pyrrolidine-2 -carboxylic acid amide (1621)

Compound **1621** was synthesized by the following steps:

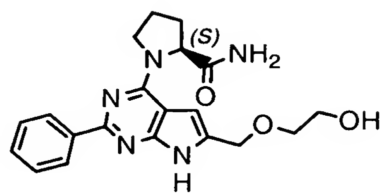


Aryl chloride **20** (3g, 10.7 mmol), DMSO (50ml) and (S)-prolinamide were combined and heated to 85°C under argon. After stirring overnight (14hrs), the mixture was cooled to room temperature and poured into 800ml of water. This was extracted with three 200ml portions of EtOAc. The combined organic layers were thoroughly washed with water (3 x 300 ml), brine, dried over $MgSO_4$, filtered and concentrated to give a dark brown solid. The solid was recrystallized twice from EtOAc to yield 1.95g (57%) of a tan solid (**1621**). ^{1}HNMR(DMSO-d_6) d 1.8-2.2 (m, 4H), 2.3 (s, 3H), 3.8 (m, 1H), 4.0 (m, 1H), 4.6 (d, 1H) 6.2 (s, 1H), 6.9 (s, 1H), 7.2 (m, 3H), 7.3 (s, 1H), 8.4 (m, 2H), 11.5 (s, 1H); MS (ES): 322 (M^++1)

Example 29: Synthesis of 1-[6-(2-Hydroxy-ethoxymethyl) -2-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-pyrrolidine-2-carboxylic acid amide(1623)

Compound **1623** was synthesized in a manner similar to that of

Example 17 using synthesis scheme IX with L-prolineamide and ethane-1,2-diol to obtain:

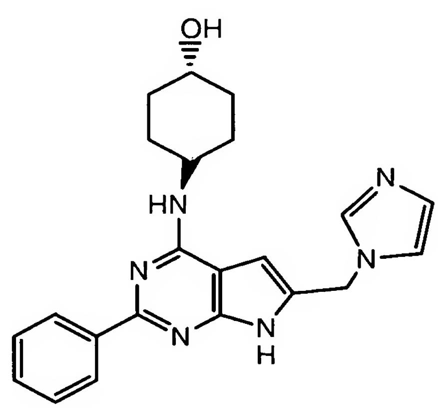


1623

MS (ES): 382 (M^{+}+1).

Example 30: Synthesis of 4-(6-Imidazol-1-ylmethyl-2-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino)-cyclohexanol (1624).

Compound **1624** was synthesized in a manner similar to that of Example 17 using synthesis scheme IX with N-6 amino cyclohexanol and imidazole to obtain:

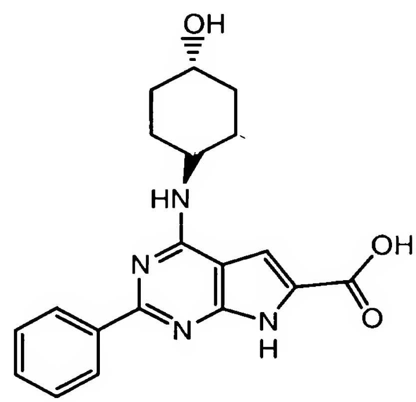


1624

MS (ES): 389 (M^{+}+1)

Example 31: Synthesis of 4-(4-Hydroxy-cyclohexylamino) -2-phenyl-7H-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid (1625)

Compound **1625** was synthesized in a manner similar to that of Example 27 using synthesis scheme IX with N-6 amino cyclohexanol to obtain:

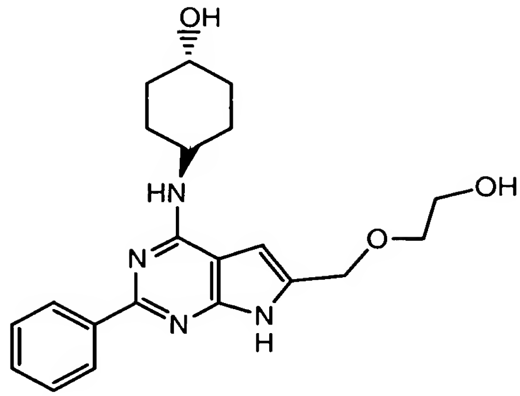


1625

MS (ES): 353 (M^{+}+1)

Example 32: Synthesis of 4-[6-(2-Hydroxy-ethoxymethyl) -2-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamino]-cyclohexanol (1626)

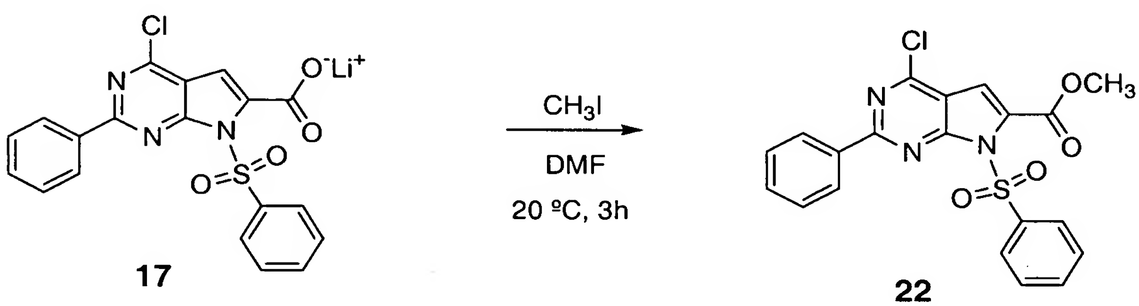
Compound **1626** was synthesized in a manner similar to that of Compound **1623** using synthesis scheme IX with N-6 amino cyclohexanol to obtain:



1626

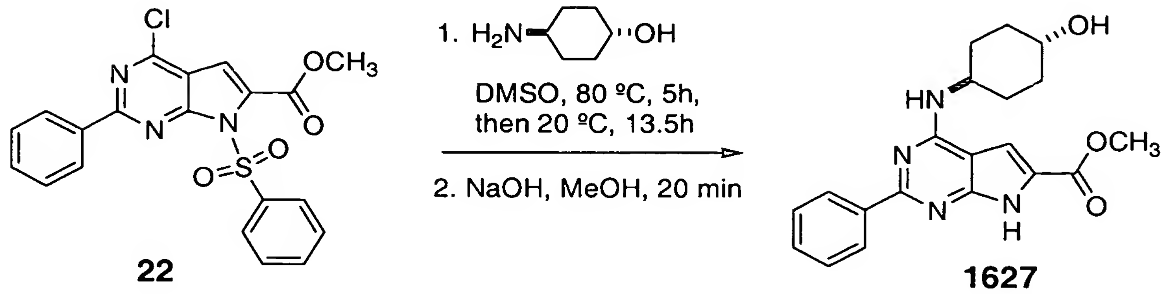
MS (ES): 383 (M^{+}+1)

Example 33: Synthesis of 4-(4-Hydroxy-cyclohexylamino) -2-phenyl-7H-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid methyl ester (1627)



17 **22**

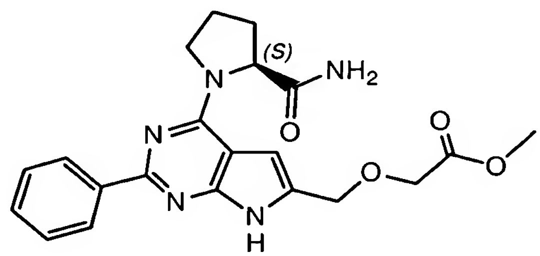
A solution of the lithium salt **17** (0.13mmol) in dry DMF (4mL) is stirred with methyl iodide (0.1mL, 1.6mmol) at 20 ºC under argon for 3h. DMF is evaporated, and aqueous ammonium chloride solution is added (15mL). The mixture is extracted with EtOAc (3´15mL), the combined organic layers are washed with water (2´10mL) and brine (10mL) and are dried over $MgSO_4$. Filtration and concentration gives 21mg (38%) of the methyl ester **22**.



A solution of the methyl ester **22** (24.5mg, 0.057mmol) and 4-*trans*-aminocyclohexanol (66mg, 0.57mmol) in DMSO (1.5mL) is heated under nitrogen to 80 ºC for 5h, then the heating is stopped, and stirring at 20 ºC is continued for 13.5h. 4% aq. acetic acid (10mL) is added to the cooled solution, and the mixture is extracted with EtOAc (3´10mL). The combined organic layers are washed with 4% aq. acetic acid (10mL), water (10mL) 2N NaOH (10mL), water (10mL), and brine (10mL) and are dried over $MgSO_4$. To a solution of the crude material obtained after filtration and concentration (1H NMR indicates about 50% removal of the benzenesulfonyl group) in THF (2mL) is added a solution of NaOH in MeOH (0.5mL of 5M solution, 2.5mmol) at ambient temperature. After 20min, water and sat. $NaHCO_3$ solution (5mL each) are added, and the mixture is extracted with EtOAc (4´15mL). The combined organic layers are washed with 2N NaOH (10mL), water (10mL), and brine (10mL) and are dried over $MgSO_4$. Chromatography of the crude material obtained after filtration and concentration on silica gel, eluting with hexanes/EtOAc 1:1 ® 1:2 yields 8.6mg (41%) of **1627** as a white solid, mp. 225-227 ºC. ^{1}H-NMR (CD_3OD): d = 1.38-1.62 (m, 4H), 1.95-2.10 (m, 2H), 2.10-2.25 (m, 2H), 3.55-3.70 (m, 1H), 3.91 (s, 3H), 4.20-4.35 (m, 1H), 7.32 (s, 1H), 7.35-7.47 (m, 3H), 8.35-8.42 (m, 2H); IR (solid): n = 3352 cm^{-1}, 3064, 2935, 2860, 1701, 1605, 1588, 1574, 1534, 1447, 1386, 1333, 1263, 1206, 1164, 1074, 938, 756, 705; MS (ES): 367 (MH^+).

Example 34: Synthesis of [4-(2-Carbamoyl-pyrrolidin-1-yl)-2-phenyl-7H-pyrrolo[2,3-d]pyrimidin-6-ylmethoxy]-acetic acid methyl ester (1628)

Compound **1628** was synthesized in a manner similar to example 26 using precursor compound **12** to obtain:

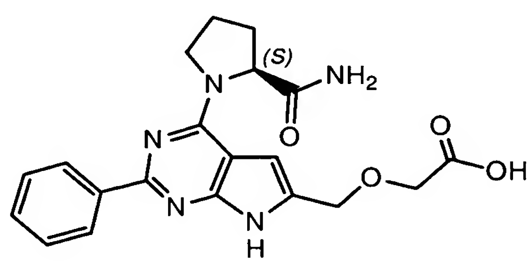


1628

MS (ES): 410 (M^{+}+1)

Example 35: Synthesis of [4-(2-Carbamoyl-pyrrolidin-1-yl)-2-phenyl-7H-pyrrolo[2,3-d]pyrimidin-6-ylmethoxy]-acetic acid (1629)

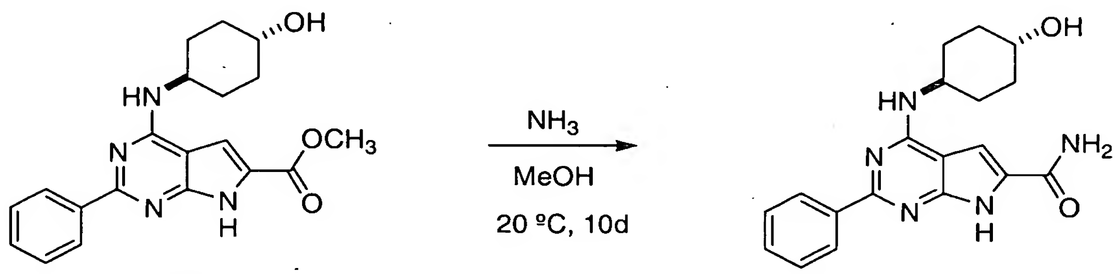
Compound **1629** was synthesized in a manner similar to compound **1628** wherein the methyl ester group was hydrolized with a base to obtain:



1629

MS (ES): 396 (M^{+}+1)

Example 36: Synthesis of 4-(4-Hydroxy-cyclohexylamino)-2-phenyl-7H-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid amide (1630)



23 **1630**

Gaseous ammonia is condensed into a solution of the pyrrolopyrimidine **23** (7.8mg, 0.021mmol) in methanol (6mL), cooled by dry ice/acetone, until a total volume of 12mL is reached. After stirring for 10d at 20 ºC, the solvents are evaporated, and the residue is purified by preparative TLC on silica gel, eluting with 5% MeOH in CH_2Cl_2. The material thus obtained is triturated with ether to yield 6.5mg (88%) of the amide **1630** as white solid, mp. 210-220 ºC (decomp.). ^{1}H-NMR (CD_3OD): d = 1.40-1.60 (m, 4H), 2.00-2.15 (m, 2H), 2.15-2.25 (m, 2H), 3.55-3.70 (m, 1H), 4.20-4.35 (m, 1H), 7.16 (s, 1H), 7.35-7.47 (m, 3H), 8.34-8.40 (m, 2H); IR (solid): n = 3358 cm^{-1}, 3064, 3025, 2964, 2924, 2853, 1652, 1593, 1539, 1493, 1452, 1374, 1326, 1251, 1197, 1113, 1074, 1028, 751, 699; MS (ES): 352 (MH^+).

<u>Activity of Compounds</u>

Adenosine 1 (A_1) receptor subtype saturation and competition radio ligand binding were carried out for compounds 1601, 1602, 1605, 1606, 1611, 1614, 1619, 1621, 1623, 1624, 1625, 1626, 1627, 1628, 1629, 1630 and 1631 as described herein and *inter alia*, on pages 192-193 of this specification. All of the above-referenced compounds equaled or surpassed the A_1 receptor binding affinity of reference compounds 1318 or 1319 as described herein and, *inter alia*, in Table 13, on pages 209-212 of the specification.

The water solubilities of the above compounds listed in Table 1 are expected to be better than reference compounds 1318 or 1319 due to their cLogP values, which were calculated using the computer program CS ChemDraw, ChemDraw Ultra ver. 6.0 ©1999 as provided by CambridgeSoft Corporation, 100 Cambridge Park Drive, Cambridge, MA 02140.

The compounds specific to the A_1 receptor listed in Table 1 had lower cLogP values, between about 1.5 to about 3.4, as compared to reference compounds 1318 or 1319 with a cLogP value about 3.8. It was not predicted that the more polar A_1 receptor compounds listed in Table 1 having lower cLogP values than the reference compounds 1318 or 1319 would still retain the potency and A_1 receptor binding selectivity as compared to those reference compounds.

Table 1

Compound	cLogP
1601	4.1
1602	3.0
1605	2.88
1606	2.1
1611	2.9
1614	1.5
1619	2.7
1621	3.37
1623	2.4
1624	2.8
1625	3.1
1626	2.8
1627	3.4
1628	2.4
1629	2.2
1630	2.4
1631	2.05

Yeast β-Galactosidase reporter gene assays for human adenosine A_1 and A_{2a} receptor: Yeast strains (S. cerevisiae) were transformed with human adenosine A_1 (A_1R; CADUS strain CY12660) or human A_{2a} (A_{2a}; CADUS strain CY8362) and the addition of a lacZ(β-Galactosidase) reporter gene to utilize as a functional readout. A complete description of the transformations is listed below (see Yeast Strains). NECA (5'-N-ethylcarboxamidoadenosine), a potent adenosine receptor agonist with similar affinity for A_1 and A_{2a} receptors, was used as a ligand for all assays. Test compounds were examined at 8 concentrations (0.1 - 10,000 nM) for ability to inhibit NECA-induced β-Galactosidase activity by CY12660 or CY8362.

Preparation of Yeast Stock Cultures: Each of the respective yeast strains, CY12660 and CY8362, were streaked onto an LT agar plate and incubated at 30°C until colonies were observed. Yeast from these colonies were added to LT liquid (pH 6.8) and grown overnight at 30°C. Each yeast strain was then diluted to an OD_{600} = 1.0-2.0 (approximately 1-2 X 10^7 cells/ml), as determined spectrophotometrically (Molecular Devices VMAX). For each 6 ml of yeast liquid culture, 4 ml of 40% glycerol (1.5:1 vol:vol) was added ("yeast/glycerol stock"). From this yeast/glycerol stock, ten 1 ml aliquots were prepared and stored at -80°C until required for assay.

Yeast A_1 R and $A_{2a}R$ Assay: One vial each of CY8362 and CY12660 yeast/glycerol stock was thawed and used to inoculate Supplemented LT liquid media, pH 6.8 (92 ml LT liquid, to which is added: 5 ml of 40% glucose, 0.45 ml of 1M KOH and 2.5 ml of Pipes, pH 6.8). Liquid cultures were grown 16-18 hr (overnight) at 30°C. Aliquots from overnight cultures were then diluted in LT media, containing 4U/ml adenosine deaminase (Type VI or VII from calf intestinal mucosa, Sigma), to obtain OD_{600} = 0.15 (1.5 X 10^6 cells/ml) for CY8362 (A2aR) and OD_{600} = 0.50 (5X10^6 cells/ml) for CY12660 (A_1R).

Assays were conducted with a final volume of 100 ul in 96-well microtiter plates, such that a final concentration of 2% DMSO was achieved in all wells. For primary screening, 1-2 concentrations of test compounds were utilized (10 uM, 1μM). For compound profiling, 8 concentrations were tested (10000, 1000, 500, 100, 50, 10, 1 and 0.1 nM). To each microtiter plate, 10 ul of 20% DMSO was added to "Control" and "Total" wells while 10 ul of Test Compound (in 20% DMSO) was added to "Unknown" wells. Subsequently, 10 ul of NECA (5 uM for A_1R, 1 uM for $A_{2a}R$) were added to "Total" and "Unknown" wells; 10 ul of PBS was added to the "Control" wells. In the final addition, 80 ul of yeast strain, CY8362 or CY12660, were added to all wells. All plates were then agitated briefly (LabLine orbital shaker 2-3 min) and allowed to incubate for 4 hrs. at 30°C in a dry oven.

β-Galactosidase activity can be quantitated using either colorimetric (e.g., ONPG, CPRG), luminescent (e.g., Galacton-Star) or fluorometric substrates (e.g., FDG, Resorufin) substrates. Currently, fluorescence detection is preferred on the basis of superior signal:noise ratio, relative freedom from interference and low cost. Fluorescein digalactopyranoside (FDG, Molecular Probes or Marker Gene Technologies), a fluorescent β-Galactosidase substrate, was added to all wells at 20 ul/well (final concentration = 80 uM). Plates were shaken for 5-6 sec (LabLine orbital shaker) and then incubated at 37°C for 90 min (95% O_2/5% CO_2 incubator). At the end of the 90 min incubation period, β-Galactosidase activity was stopped using 20 ul/well of 1M Na_2CO_3 and all plates shaken for 5-6 sec. Plates were then agitated for 6 sec and relative fluorescence intensity determined using a fluorometer (Tecan Spectrafluor; excitation = 485 nm, emission = 535 nm).

Calculations: Relative fluorescence values for "Control" wells

were interpreted as background and subtracted from "Total" and "Unknown" values. Compound profiles were analyzed via logarithmic transformation (x-axis: compound concentration) followed by one site competition curve fitting to calculate IC_{50} values (GraphPad Prism).

Yeast strains: Saccharomyces cerevisiae strains CY12660 [far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1::LYS2 ste3*1156 gpa1(41)-Gαi3 lys2 ura3 leu2 trp1: his3; LEU2 PGKp-Mfα1Leader-hA1R-PHO5term 2mu-orig REP3 Ampr] and CY8362 [gpa1p-rGαsE10K far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1: LYS2 ste3*1156 lys2 ura3 leu2 trp1 his3; LEU2 PGKp-hA2aR 2mu-ori REP3 Ampr] were developed.

LT Media: LT (Leu-Trp supplemented) media is composed of 100g DIFCO yeast nitrogen base, supplemented with the following: 1.0g valine, 1.0g aspartic acid, 0.75g phenylalanine, 0.9g lysine, 0.45g tyrosine, 0.45g isoleucine, 0.3g methionine, 0.6g adenine, 0.4g uracil, 0.3g serine, 0.3g proline, 0.3g cysteine, 0.3g arginine, 0.9g histidine and 1.0g threonine.

Construction of Yeast Strains Expressing Human A_1 Adenosine Receptor

In this example, the construction of yeast strains expressing a human A_1 adenosine receptor functionally integrated into the yeast pheromone system pathway is described.

I. Expression Vector Construction

To construct a yeast expression vector for the human A_1 adenosine receptor, the A_1 adenosine receptor cDNA was obtained by reverse transcriptase PCR of human hippocampus mRNA using primers designed based on the published sequence of the human A_1 adenosine receptor and standard techniques. The PCR product was subcloned into the NcoI and XbaI sites of the yeast expression plasmid pMP15.

The pMP15 plasmid was created from pLPXt as follows: The XbaI site of YEP51 (Broach, J.R. et al. (1983) "Vectors for high-level, inducible expression of cloned genes in yeast" p. 83-117 in M. Inouye (ed.), Experimental Manipulation of Gene Expression. Academic Press, New York) was eliminated by digestion, end-fill and religation to create Yep51NcoDXba. Another XbaI site was created at the BamHI site by digestion with BamHI, end-fill, linker (New England Biolabs, # 1081) ligation, XbaI digestion and re-ligation to generate YEP51NcoXt. This plasmid was digested with Esp31 and NcoI and ligated to Leu2 and PGKp fragments generated by PCR. The 2 kb Leu2 PCR product was generated by amplification from YEP51Nco using primers containing Esp31 and BglII sites. The 660 base pair PGKp PCR product was generated by amplification from pPGKαs (Kang, Y.-S. et al. (1990) Mol. Cell. Biol. 10:2582-2590) with PCR primers containing BglII and NcoI sites. The resulting plasmid is called pLPXt. pLPXt was modified by inserting the coding region of the a-factor prepro leader into the NcoI site. The prepro leader was inserted so that the NcoI cloning site was maintained at the 3′ end of the leader, but not regenerated at the 5′ end. In this way receptors can be cloned by digestion of the plasmid with NcoI and XbaI. The resulting plasmid is called pMP15.

The pMP15 plasmid into which was inserted the human A_1 adenosine receptor cDNA was designated p5095. In this vector, the receptor cDNA is fused to the 3′ end of the yeast a-factor prepro leader. During protein maturation the prepro peptide sequences are cleaved to generate mature full-length receptor. This occurs during processing of the receptor through the yeast secretory pathway. This plasmid is maintained by Leu selection (i.e., growth on medium lacking leucine). The sequence of the cloned coding region was determined and found to be equivalent to that in the published literature (GenBank accession numbers S45235 and S56143).

II. Yeast Strain Construction

To create a yeast strain expressing the human A_1 adenosine receptor, yeast strain CY7967 was used as the starting parental strain. The genotype of CY7967 is as follows:

MATα gpaD1163 gpa1(41)Gαi3 far1D1442 tbt-1 FUS1-HIS3 can1 ste14::trp1::LYS2 ste3D1156 lys2 ura3 leu2 trp1 his3

The genetic markers are reviewed below:

Table 2

MATa..........	Mating type **a**.
gpa1D1163..........	The endogenous yeast G-protein GPA1 has been deleted.
gpa1(41)Gαi3........	gpa1(41)-Gai3 was integrated into the yeast genome. This chimeric Ga protein is composed of the first 41 amino acids of the endogenous yeast Ga subunit GPA1 fused to the mammalian G-protein Gai3 in which the cognate N-terminal amino acids have been deleted.
far1D1442...........	FAR1 gene (responsible for cell cycle arrest) has been deleted (thereby preventing cell cycle arrest upon activation of the pheromone response pathway).
tbt-1..................	strain with high transformation efficiency by electroporation.
FUS1-HIS3.........	a fusion between the FUS1 promoter and the HIS3 coding region (thereby creating a pheromone inducible HIS3 gene).
can 1..................	arginine/canavinine permease.
ste14::trp1::LYS2....	gene disruption of STE14, a C-farnesyl methyltransferase (thereby lowering basal signaling through the pheromone pathway).
ste3D1156............	endogenous yeast STR, the a factor pheromone receptor (STE3) was disrupted.
lys2..................	defect in 2-aminoapidate reductase, yeast need lysine to grow.
ura3..................	defect in orotidine-5'-phosphate decarboxylase, yeast need uracil to grow
leu2..................	defect in b-isopropylmalate dehydrogenase, yeast need leucine to grow.
trp1..................	defect in phosphoribosylanthranilate, yeast need tryptophan to grow.
his3..................	defect in imidazoleglycerolphosphate dehydrogenase, yeast need histidine to grow.

Two plasmids were transformed into strain CY7967 by electroporation: plasmid p5095 (encoding human A_1 adenosine receptor; described above) and plasmid p1584, which is a FUS1-β-galactosidase reporter gene plasmid. Plasmid p1584 was derived from plasmid pRS426 (Christianson, T.W. et al. (1992) Gene 110:119-1122). Plasmid pRS426 contains a polylinker site at nucleotides 2004-2016. A fusion between the FUS1 promoter and the β-galactosidase gene was inserted at the restriction sites EagI and XhoI to create plasmid p1584. The p1584 plasmid is maintained by Trp selection (i.e., growth on medium lacking leucine).

The resultant strain carrying p5095 and p1584, referred to as CY12660, expresses the human A_1 adenosine receptor. To grow this strain in liquid or on agar plates, minimal media lacking leucine and tryptophan was used. To perform a growth assay on plates (assaying FUS1-HIS3), the plates were at pH 6.8 and contained 0.5-2.5 mM 3-amino-1,2,4-triazole and lacked leucine, tryptophan and histidine. As a control for specificity, a comparison with one or more other yeast-based seven transmembrane receptor screens was included in all experiments.

Construction of Yeast Strains Expressing Human A2a Adenosine Receptor

In this example, the construction of yeast strains expressing a human A_{2a} adenosine receptor functionally integrated into the yeast pheromone system pathway is described.

I. Expression Vector Construction

To construct a yeast expression vector for the human A2a adenosine receptor, the human A2a receptor cDNA was obtained from Dr. Phil Murphy (NIH). Upon receipt of this clone, the A2a receptor insert was sequenced and found to be identical to the published sequence (GenBank accession # S46950). The receptor cDNA was excised from the plasmid by PCR with VENT

polymerase and cloned into the plasmid pLPBX, which drives receptor expression by a constitutive Phosphoglycerate Kinase (PGK) promoter in yeast. The sequence of the entire insert was once again sequenced and found to be identical with the published sequence. However, by virtue of the cloning strategy employed there were three amino acids appended to the carboxy-terminus of the receptor, GlySerVal.

II. Yeast Strain Construction

To create a yeast strain expressing the human A2a adenosine receptor, yeast strain CY8342 was used as the starting parental strain. The genotype of CY8342 is as follows: MATa far1D1442 tbt1-1 lys2 ura3 leu2 trp1 his3 fus1-HIS3 can1 ste3D1156 gpaD1163 ste14::trp1::LYS2 gpa1p-r$G_{\alpha s}$E10K (or gpa1p-r$G_{\alpha s}$D229S or gpa1p-r$G_{\alpha s}$E10K+D229S)

The genetic markers are as described above, except for the G-protein variation. For human A2a receptor-expression, yeast strains were utilized in which the endogenous yeast G protein GPA1 had been deleted and replaced by a mammalian $G_{\alpha s}$. Three rat $G_{\alpha s}$ mutants were utilized. These variants contain one or two point mutations which convert them into proteins which couple efficiently to yeast $\beta\gamma$. They are identified as $G_{\alpha s}$E10K (in which the glutamic acid at position ten is replaced with lysine), $G_{\alpha s}$D229S (in which the aspartic acid at position 229 is replaced with serine) and $G_{\alpha s}$E10K+D229S (which contains both point mutations).

Strain CY8342 (carrying one of the three mutant rat $G_{\alpha s}$ proteins) was transformed with either the parental vector pLPBX (Receptor$^-$) or with pLPBX-A2a (Receptor$^+$). A plasmid with the FUS1 promoter fused to β-galactosidase coding sequences (described in above) was added to assess the magnitude of activation of the pheromone response pathway.

Functional Assay using Yeast Strains Expressing Human A_1 Adenosine Receptor

In this example, the development of a functional screening assay in yeast for modulators of the human A_1 adenosine receptor is described.

I. Ligands Used in Assay

Adenosine, a natural agonist for this receptor, as well as two other synthetic agonists were utilized for development of this assay. Adenosine, reported to have an EC_{50} of approximately 75 nM, and (-)-N6-(2-phenylisopropyl)-adenosine (PIA) with a reported affinity of approximately 50 nM were used in a subset of experiments. 5'-N-ethylcarboxamido-adenosine (NECA) was used in all growth assays. To prevent signaling due to the presence of adenosine in the growth media, adenosine deaminase (4U/ml) was added to all assays.

II. Biological Response in Yeast

The ability of the A_1 adenosine receptor to functionally couple in a heterologous yeast system was assessed by introducing the A_1 receptor expression vector (p5095, described above) into a series of yeast strains that expressed different G protein subunits. The majority of these transformants expressed G_α subunits of the $G_{\alpha i}$ or $G_{\alpha o}$ subtype. Additional G_α proteins were also tested for the possible identification of promiscuous receptor-$G\alpha$ protein coupling. In various strains, a STE18 or a chimeric STE18-$G\gamma2$ construct was integrated into the genome of the yeast. The yeast strains harbored a defective HIS3 gene and an integrated copy of FUS1-HIS3, thereby allowing for selection in selective media containing 3-amino-1,2,4-triazole (tested at 0.2, 0.5 and 1.0 mM) and lacking histidine. Transformants were isolated and monolayers were prepared on media containing 3-amino-1,2,4-triazole, 4 U/ml adenosine deaminase and lacking histidine. Five microliters of various concentrations of

ligand (e.g., NECA at 0, 0.1, 1.0 and 10 mM) was applied. Growth was monitored for 2 days. Ligand-dependent growth responses were tested in this manner in the various yeast strains. The results are summarized in Table 3 below. The symbol (-) indicates that ligand-dependent receptor activation was not detected while (+) denotes ligand-dependent response. The term "LIRMA" indicates ligand independent receptor mediated activation.

Table 3

Yeast strain	Gα subunit	Gγ subunit	Strain Variants	Result
CY1316	GPA_1	STE18		–
	$GPA41\text{-}G_{\alpha i1}$			+
	$GPA41\text{-}G_{\alpha i2}$			+
	$GPA41\text{-}G_{\alpha i3}$			+
	$GPA41\text{-}G_{ai2}\text{-}G_{\alpha OB}$			LIRMA
	$GPA41\text{-}G_{\alpha SE10K}$			–
	$GPA41\text{-}G_{\alpha SD229S}$			–
CY7967	$GPA41\text{-}G_{\alpha i3}$-integrated	STE18		+++
CY2120	GPA_1	STE18	sst2Δ	+
	$GPA41\text{-}G_{\alpha i1}$			+
	$GPA41\text{-}G_{\alpha i2}$			+
	$GPA41\text{-}G_{\alpha i3}$			+
	$GPA41\text{-}G_{ai2}\text{-}G_{\alpha OB}$			LIRMA
	$GPA41\text{-}G_{\alpha SE10K}$			–
	$GPA41\text{-}G_{\alpha SD229S}$			–
CY9438	GPA_1	STE18-Gγ2		–
	$GPA41\text{-}G_{\alpha i1}$			+
	$GPA41\text{-}G_{\alpha i2}$			+
	$GPA41\text{-}G_{\alpha i3}$			+
	$GPA41\text{-}G_{ai2}\text{-}G_{\alpha OB}$			LIRMA
	$GPA41\text{-}G_{\alpha SE10K}$			–
	$GPA41\text{-}G_{\alpha SD229S}$			–
CY10560	GPA_1-integrated	STE18-Gγ2	sst2Δ	++

As indicated in Table 3, the most robust signaling was found to occur in a yeast strain expressing the $GPA_1(41)\text{-}G_{\alpha i3}$ chimera.

III. fus1-LacZ Assay

To characterize activation of the pheromone response pathway more fully, synthesis of β-galactosidase through fus1LacZ in response to agonist stimulation was measured. To perform the β-galactosidase assay, increasing concentrations of ligand were added to mid-log culture of human A_1 adenosine receptor

expressed in a yeast strain co-expressing a Ste18-Gγ2 chimera and GPA$_{41}$-G$_{\alpha i3}$. Transformants were isolated and grown overnight in the presence of histidine and 4 U/ml adenosine deaminase. After five hours of incubation with 4 U/ml adenosine deaminase and ligand, induction of β-galactosidase was measured using CPRG as the substrate for β-galactosidase. 5×10^5 cells were used per assay.

The results obtained with NECA stimulation indicated that at a NECA concentration of 10^{-8} M approximately 2-fold stimulation of β-galactosidase activity was achieved. Moreover, a stimulation index of approximately 10-fold was observed at a NECA concentration of 10^{-5} M.

The utility of this assay was extended by validation of the activity of antagonists on this strain. Two known adenosine antagonist, XAC and DPCPX, were tested for their ability to compete against NECA (at 5 mM) for activity in the β-galactosidase assay. In these assays, β-galactosidase induction was measured using FDG as the substrate and 1.6×10^5 cells per assay. The results indicated that both XAC and DPCPX served as potent antagonists of yeast-expressed A_1 adenosine receptor, with IC_{50} values of 44 nM and 49 nM, respectively.

In order to determine if this inhibitory effect was specific to the A_1 subtype, a series of complementary experiments were performed with the yeast-based A_{2a} receptor assay. Results obtained with the A_{2a} yeast-based assay indicated that XAC was a relatively effective A_{2a} receptor antagonist, consistent with published reports. In contrast, DPCPX was relatively inert at this receptor, as expected from published reports.

IV. Radioligand Binding

The A_1 adenosine receptor assay was further characterized by measurement of the receptor's radioligand binding parameters. Displacement binding of [^{3}H]CPX by several adenosine receptor reference compounds, XAC, DPCPX, and CGS, was analyzed using membranes prepared from yeast expressing the human A_1 adenosine receptor. The results with yeast membranes expressing the human A_1 adenosine receptor were compared to those from yeast membranes expressing the human A2a adenosine receptor or the human A3 receptor to examine the specificity of binding. To perform the assay, fifty mg of membranes were incubated with 0.4 nM [^{3}H]CPX and increasing concentrations of adenosine receptor ligands. Incubation was in 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$, 0.25 % BSA and 2 U/ml adenosine deaminase in the presence of protease inhibitors for 60 minutes at room temperature. Binding was terminated by addition of ice-cold 50 mM Tris-HCl, pH 7.4 plus 10 mM $MgCl_2$, followed by rapid filtration over GF/B filters previously soaked with 0.5 % polyethyenimine, using a Packard 96-well harvester. Data were analyzed by nonlinear least square curve fitting procedure using Prism 2.01 software. The IC_{50} values obtained in this experiment are summarized in Table 4, below:

Table 4

		IC_{50} [nM]	
Compound	hA1R	hA2aR	hA3R
XAC	6.6	11.7	53.1
DPCPX	8.5	326.4	1307.0
CGS-15943	13.1	15.8	55.5
NECA	215.5	294.9	34.9
R-PIA	67.6	678.1	23.6
IB-MECA	727.7	859.4	3.1
Alloxozine	1072.0	1934.0	8216.0

These data indicate that the reference compounds have affinities consistent with those reported in the literature. The data further indicate that the yeast-based assays are of

sufficient sensitivity to discriminate receptor subtype specificity.

Functional Assay using Yeast Strains Expressing Human A2a Adenosine Receptor

In this example, the development of a functional screening assay in yeast for modulators of the human A_1 adenosine receptor is described.

I. Ligands Used in Assay

The natural ligand adenosine, as well as other thoroughly characterized and commercially available ligands were used for study of the human A2a receptor functionally expressed in yeast. Three ligands have been used in the establishment of this assay. They include:

Ligand	Reported K_i	Function
Adenosine	500 nM	agonist
5'-N-ethylcarboxamidoadenosine	10-15 nM	agonist
(NECA) (-)-N6-(2-phenylisopropyl)-adenosine (PIA)	100-125 nM	agonist

To prevent signaling due to the presence of adenosine in the growth media, adenosine deaminase (4U/ml) was added to all assays.

II. Biological Response in Yeast

A2a receptor agonists were tested for the capacity to stimulate the pheromone response pathway in yeast transformed with the A2a receptor expression plasmid and expressing either $G_{\alpha s}$E10K, $G_{\alpha s}$D229S or $G_{\alpha s}$E10K+D229S. The ability of ligand to stimulate the pheromone response pathway in a receptor dependent manner was indicated by an alteration in the yeast phenotype. Receptor activation modified the phenotype from

histidine auxotrophy to histidine prototrophy (activation of fus1-HIS3). Three independent transformants were isolated and grown overnight in the presence of histidine. Cells were washed to remove histidine and diluted to 2×10^6 cells/ml. 5 µl of each transformant was spotted onto nonselective media (including histidine) or selective media (1 mM AT) in the absence or presence of 4 U/ml adenosine deaminase. Plates were grown at 30 °C for 24 hours. In the presence of histidine both Receptor$^+$ (R^+) and Receptor$^-$ (R^-) strains were capable of growth. However, in the absence of histidine only R^+ cells grew. Since no ligand had been added to these plates two explanations were possible for this result. One possible interpretation was that the receptor bearing yeast were at a growth advantage due to Ligand Independent Receptor Mediated Activation (LIRMA). Alternatively the yeast could have been synthesizing the ligand adenosine. To distinguish between these two possibilities, an enzyme which degrades the ligand, adenosine deaminase (ADA), was added to the growing yeast and plates. In the presence of adenosine deaminase R^+ cells no longer grew in the absence of histidine, indicating that the yeast were indeed synthesizing ligand.

This interpretation was confirmed by an A2a growth assay in liquid. In this experiment R^+ yeast (a $G_{\alpha s}$E10K strain expressing the A2a receptor) were inoculated at three densities (1×10^6 cell/ml; 3×10^5 cells/ml; or 1×10^5 cells/ml) in the presence or absence of adenosine deaminase (4 U/ml). The stringency of the assay was enhanced with increasing concentrations (0, 0.1, 0.2 or 0.4 mM)of 3-amino-1,2,4-triazole (AT), a competitive antagonist of imidazoleglycerol-P dehydratase, the protein product of the HIS3 gene. In the presence of adenosine deaminase and 3-amino-1,2,4-triazole yeast grew less vigorously. However in the absence of 3-amino-1,2,4-triazole, adenosine deaminase had little effect. Thus adenosine deaminase itself had no direct

effect upon the pheromone response pathway.

An alternative approach to measuring growth and one that can be miniaturized for high throughput screening is an A2a receptor ligand spot assay. A $G_{\alpha s}$E10K strain expressing the A2a receptor (A2aR+) or lacking the receptor (R-) was grown overnight in the presence of histidine and 4 U/ml adenosine deaminase. Cells were washed to remove histidine and diluted to 5 x 10^6 cells/ml. 1 x 10^6 cells were spread onto selective plates containing 4 U/ml adenosine deaminase and 0.5 or 1.0 mM 3-amino-1,2,4-triazole (AT) and allowed to dry for 1 hour. 5 µl of the following reagents were applied to the monolayer: 10 mM adenosine, 38.7 mM histidine, dimethylsulfoxide (DMSO), 10 mM PIA or 10 mM NECA. Cells were grown 24 hours at 30°C. The results showed that cells without receptor could only grow when histidine was added to the media. In contrast, R^+ cells only grew in areas where the A2a receptor ligands PIA and NECA had been spotted. Since the plates contained adenosine deaminase, the lack of growth where adenosine had been spotted confirmed that adenosine deaminase was active.

III. fus1 LacZ Assay

To quantitate activation of the yeast mating pathway, synthesis of β-galactosidase through *fus1*LacZ was measured. Yeast strains expressing $G_{\alpha s}$E10K, $G_{\alpha s}$D229S or $G_{\alpha s}$E10K+D229S were transformed with a plasmid encoding the human A2a receptor (R+) or with a plasmid lacking the receptor (R-). Transformants were isolated and grown overnight in the presence of histidine and 4 U/ml adenosine deaminase. 1 x 10^7 cells were diluted to 1 x 10^6 cells/ml and exposed to increasing concentrations of NECA for 4 hours, followed by determination of the β-galactosidase activity in the cells. The results demonstrated that essentially no β-galactosidase activity was detected in R- strains, whereas increasing amounts of β-galactosidase activity were detected in R+

strains expressing either $G_{\alpha s}$E10K, $G_{\alpha s}$D229S or $G_{\alpha s}$E10K+D229S as the concentration of NECA increased, indicating a dose dependent increase in units of β-galactosidase detected in response to exposure to increased ligand concentration. This dose dependency was only observed in cells expressing the A2a receptor. Furthermore the most potent $G_{\alpha s}$ construct for the A2a receptor was $G_{\alpha s}$E10K. The $G_{\alpha s}$D229S construct was the second-most potent $G_{\alpha s}$ construct for the A2a receptor, while the $G_{\alpha s}$E10K+D229S construct was the least potent of the three $G_{\alpha s}$ constructs tested, although even the $G_{\alpha s}$E10K+D229S construct stimulated readily detectable amounts of β-galactosidase activity.

For a further description of the assays identified, see International Application No. WO 99/63099, entitled "Functional Expression of Adenosine Receptors in Yeast", published December 9, 1999, the entire contents of which are hereby incorporated herein by reference.

Pharmacological Characterization of the Human Adenosine Receptor Subtypes

Material and Methods

Materials. [^{3}H]-DPCPX [Cyclopentyl-1,3-dipropylxantine, 8-[dipropyl-2,3-^{3}H(N)] (120.0 Ci/mmol); [^{3}H]-CGS 21680, [carboxyethyl-^{3}H (N)] (30 Ci/mmol) and [^{125}I] -AB-MECA ([^{125}I]-4-Aminobenzyl-5'-N-Methylcarboxamideoadenosine) (2,200 Ci/mmol) were purchased from New England Nuclear (Boston, MA). XAC (Xantine amine congener); NECA (5'-N-Ethylcarboxamidoadenosine); and IB-MECA from Research Biochemicals International (RBI, Natick, MA). The Adenosine Deaminase and Complete protease inhibitor cocktail tablets were purchased from Boehringer Mannheim Corp. (Indianapolis, IN). Membranes from HEK-293 cells stably expressing the human Adenosine 2a [RB-HA2a]; Adenosine 2b [RB-HA2b] or Adenosine

3 [RB-HA3] receptor subtypes, respectively were purchased from Receptor Biology (Beltsville, MD). Cell culture reagents were from Life Technologies (Grand Island, NY) except for serum that was from Hyclone (Logan, UT).

Yeast strains: Saccharomyces cerevisiae strains CY12660 [far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1::LYS2 ste3*1156 gpa1(41)-Gαi3 lys2 ura3 leu2 trp1: his3; LEU2 PGKp-Mfα1Leader-hA1R-PHO5term 2mu-orig REP3 Ampr] and CY8362 [gpa1p-rGαsE10K far1*1442 tbt1-1 fus1-HIS3 can1 ste14::trp1: LYS2 ste3*1156 lys2 ura3 leu2 trp1 his3; LEU2 PGKp-hA2aR 2mu-ori REP3 Ampr] were developed as described above.

Yeast culture: Transformed yeast were grown in Leu-Trp [LT] media (pH 5.4) supplemented with 2% glucose. For the preparation of membranes 250 ml of LT medium were inoculated with start titer of 1-2 x 10^6 cells/ml from a 30 ml overnight culture and incubated at 30°C under permanent oxygenation by rotation. After 16 h growth the cells were harvested by centrifugation and membranes were prepared as described below.

Mammalian Tissue Culture: The HEK-293 cells stably expressed human Adenosine 2a receptor subtype (Cadus clone # 5) were grown in Dulbeco's minimal essential media (DMEM) supplemented with 10% fetal bovine serum and 1X penicillin/streptomycin under selective pressure using 500 mg/ml G418 antibiotic, at 37°C in a humidified 5% CO_2 atmosphere.

Yeast Cell Membrane Preparations: 250 ml cultures were harvested after overnight incubation by centrifugation at 2,000 x g in a Sorvall RT6000 centrifuge. Cells were washed in ice-cold water, centrifuged at 4°C and the pellet was resuspended in 10 ml ice-cold lysis buffer [5 mM Tris-HCl, pH 7.5; 5 mM EDTA; and 5 mM EGTA] supplemented with Protease inhibitor cocktail tablets (1 tablet per 25 ml buffer). Glass

beads (17 g; Mesh 400-600; Sigma) were added to the suspension and the cells were broken by vigorous vortexing at 4 °C for 5 min. The homogenate was diluted with additional 30 ml lysis buffer plus protease inhibitors and centrifuged at 3,000 x g for 5 min. Subsequently the membranes were peleted at 36,000 x g (Sorvall RC5B, type SS34 rotor) for 45 min. The resulting membrane pellet was resuspended in 5 ml membrane buffer [50 mM Tris-HCl, pH 7.5; 0.6 mM EDTA; and 5 mM $MgCl_2$] supplemented with Protease inhibitor cocktail tablets (1 tablet per 50 ml buffer) and stored at -80 °C for further experiments.

Mammalian Cell Membrane Preparations: HEK-293 cell membranes were prepared as described previously (*Duzic E et al.: J. Biol. Chem., 267, 9844-9851, 1992*) Briefly, cells were washed with PBS and harvested with a rubber policeman. Cells were pelted at 4 °C 200 x g in a Sorvall RT6000 centrifuge. The pellet was resuspended in 5 ml/dish of lysis buffer at 4 °C (5 mM Tris-HCl, pH 7.5; 5 mM EDTA; 5 mM EGTA; 0.1 mM Phenylmethylsulfonyl fluoride, 10 mg/ml pepstatin A; and 10 mg/ml aprotinin) and homogenized in a Dounce homogenizer. The cell lysate was then centrifuged at 36,000 x g (Sorvall RC5B, type SS34 rotor) for 45 min and the pellet resuspended in 5 ml membrane buffer [50 mM Tris-HCl, pH 7.5; 0.6 mM EDTA; 5 mM $MgCl_2$; 0.1 mM Phenylmethylsulfonyl fluoride, 10 mg/ml pepstatin A; and 10 mg/ml aprotinin) and stored at -80 °C for further experiments.

The Bio-Rad protein assay kits, based on the Bradford dye-binding procedure, (Bradford, M.: *Anal. Biochem.* 72:248 (1976)) were used to determine total protein concentration in yeast and mammalian membranes.

Adenosine 1 receptor subtype saturation and competition radioligand binding: Saturation and competition binding on membranes from yeast cell transformed with human A_1 receptor

subtype were carried out using antagonist [^{3}H] DPCPX as a radioactive ligand. Membranes was diluted in binding buffer [50 mM Tris-HCl, pH 7.4; containing 10 mM $MgCl_2$; 1.0 mM EDTA; 0.25% BSA; 2 U/ml adenosine deaminase and 1 protease inhibitor cocktail tablet/50 ml] at concentrations of 1.0 mg/ml.

In saturation binding membranes (50 µg/well) were incubate with increasing concentrations of [^{3}H] DPCPX (0.05 - 25 nM) in a final volume of 100 µl of binding buffer at 25°C for 1 hr in the absence and presence of 10 µM unlabeled XAC in a 96-well microtiter plate.

In competition binding membranes (50 µg/well) were incubate with [^{3}H] DPCPX (1.0 nM) in a final volume of 100 µl of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 µM unlabeled XAC or increasing concentrations of competing compounds in a 96-well microtiter plate.

Adenosine 2a receptor subtype competition radioligand binding: Competition binding on membranes from HEK293 cell stably expressing the human A2a receptor subtype were carried out using agonist [^{3}H] CGS-21680 as a radioactive ligand. Membranes was diluted in binding buffer [50 mM Tris-HCl, pH 7.4; containing 10 mM $MgCl_2$; 1.0 mM EDTA; 0.25% BSA; 2 U/ml adenosine deaminase and 1 protease inhibitor cocktail tablet/50 ml] at concentrations of 0.2 mg/ml. Membranes (10 µg/well) were incubate with [^{3}H] CGS-21680 (100 nM) in a final volume of 100 µl of binding buffer at 25°C for 1 hr in the absence and presence of 50 µM unlabeled NECA or increasing concentrations of competing compounds in a 96-well microtiter plate.

Adenosine 3 receptor competition radioligand binding: Competition binding on membranes from HEK293 cell stably expressing the human A3 receptor subtype were carried out

using agonist [^{125}I] AB-MECA as a radioactive ligand. Membranes was diluted in binding buffer [50 mM Tris-HCl, pH 7.4; containing 10 mM $MgCl_2$; 1.0 mM EDTA; 0.25% BSA; 2 U/ml adenosine deaminase and 1 protease inhibitor cocktail tablet/50 ml] at concentrations of 0.2 mg/ml. Membranes (10 µg/well) were incubate with [^{125}I] AB-MECA (0.75 nM) in a final volume of 100 µl of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 µM unlabeled IB-MECA or increasing concentrations of competing compounds in a 96-well microtiter plate.

At the end of the incubation, the A_1, A_{2a} and A_3 receptor subtypes radioligand binding assays was terminated by the addition of ice-cold 50 mM Tris-HCl (pH 7.4) buffer supplemented with 10 mM $MgCl_2$, followed by rapid filtration over glass fiber filters (96-well GF/B UniFilters, Packard) previously presoaked in 0.5% polyethylenimine in a Filtermate 196 cell harvester (Packard). The filter plates were dried coated with 50 µl /well scintillation fluid (MicroScint-20, Packard) and counted in a TopCount (Packard). Assays were performed in triplicate. Non-specific binding was 5.6 ± 0.5%, 10.8 ± 1.4% and 15.1 ± 2.6% of the total binding in a A1R, A2aR and A3R binding assay, respectively.

Adenosine 2b receptor subtype competition radioligand binding: Competition binding on membranes from HEK293 cell stably expressing the human A2b receptor subtype were carried out using A_1 receptor antagonist [^{3}H] DPCPX as a radioactive ligand. Membranes was diluted in binding buffer [10 mM Hepes-KOH, pH 7.4; containing 1.0 mM EDTA; 0.1 mM Benzamidine and 2 U/ml adenosine deaminase] at concentrations of 0.3 mg/ml. Membranes (15 µg/well) were incubate with [^{3}H] DPCPX (15 nM) in a final volume of 100 µl of binding buffer at 25^{o}C for 1 hr in the absence and presence of 10 µM unlabeled XAC or increasing concentrations of competing compounds in a 96-well

microtiter plate. At the end of the incubation, the assay was terminated by the addition of ice-cold 10 mM Hepes-KOH (pH 7.4) buffer followed by rapid filtration over glass fiber filters (96-well GF/C UniFilters, Packard) previously presoaked in 0.5% polyethylenimine in a Filtermate 196 cell harvester (Packard). The filter plates were dried coated with 50 µl/well scintillation fluid (MicroScint-20, Packard) and counted in a TopCount (Packard). Assays were performed in triplicate. Non-specific binding was 14.3 ± 2.3% of the total binding.

Specific binding of [^{3}H] DPCPX; [^{3}H] CGS-21680 and [^{125}I] AB-MECA was defined as the difference between the total binding and non-specific binding. Percent inhibition of the compounds was calculated against total binding. Competition data were analyzed by iterative curve fitting to a one site model, and K_I values were calculated from IC_{50} values (Cheng and Prusof, Biochem. Pharmacol. 22, 3099-3109, 1973) using the GraphPad *Prizm 2.01* software.

Results

A primary function of certain cell surface receptors is to recognize appropriate ligands. Accordingly, we determined ligand binding affinities to establish the functional integrity of the Adenosine 1 receptor subtype expressed in yeast. Crude membranes prepared from *Saccharomyces cerevisiae* transformed with human Adenosine 1 receptor subtype construct exhibited specific saturable binding of [^{3}H] DPCPX with a K_D of 4.0 ± 0.19 nM. The K_D and B_{max} value were calculated from the saturation isotherm and Scatchard transformation of the data indicated a single class of binding sites. The densities of adenosine binding sites in the yeast membrane preparations were estimated to 716.8 ± 43.4 fmol/mg membrane protein.

The pharmacological subtype characteristics of the recombinant

yeast cells transformed with human A_1 receptor subtype were investigated with subtype selective adenosine ligands (XAC, DPCPX; CGS-15943; Compound 600; Compound 1002; NECA, (R)-PIA; IB-MECA and Alloxazine) that competed with [^{3}H] DPCPX in the expected rank order. Displacement curves recorded with these compounds show the typical steepness with all the ligands, and the data for each of the ligands could be modeled by a one-site fit. The apparent dissociation constants estimated for the individual compound from the curves (Table 5) are consistent with value published for the receptor obtained from other sources.

Table 5

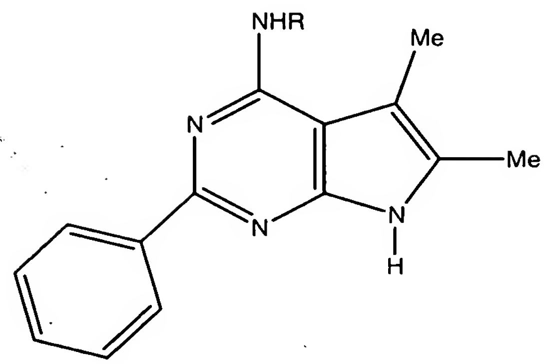
Ki values for membranes from yeast cells transformed with human A_1 receptor subtype

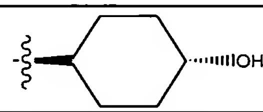
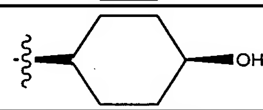
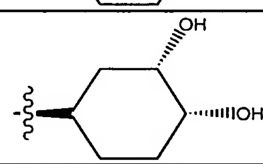
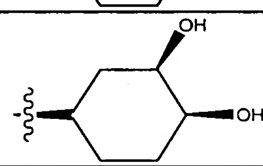
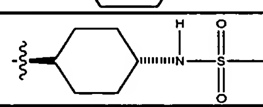
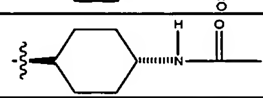
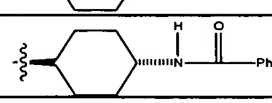
Ligands	K_I (nM)
XAC	5.5
DPCPX	7.1
CGS-1594	10.8
NECA	179.6
(R)-PIA	56.3
IB-MECA	606.5
Alloxazine	894.1
Compound 600	13.9
Compound 1002	9.8

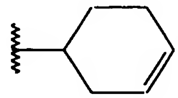
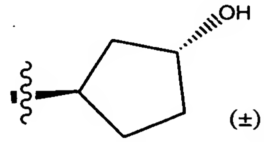
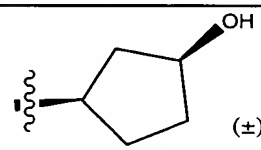
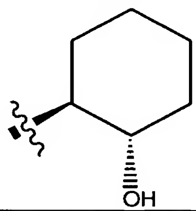
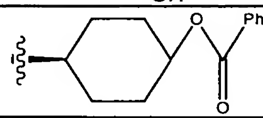
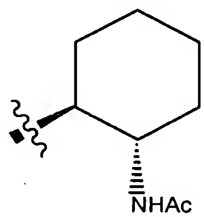
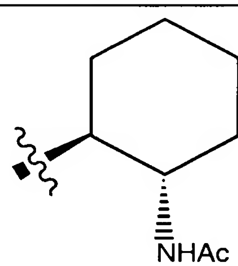
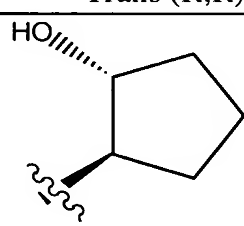
Tables 6 through 12 demonstrate the efficacy and structure activity profiles of deazapurines of the invention. Tables 13 and 14 demonstrate selectivity can be achieved for human adenosine receptor sites by modulation of the functionality about the deazapurine structure. Table 14 also demonstrates the surprising discovery that the compounds set forth therein have subnanomolar activity and higher selectivity for the A_{2b} receptor as compared to the compounds in Table 13.

TABLE 6

Effect of N_6-Substituent



		A1	
Compound	R	Binding Ki (nM)	Yeast IC50 (nM)
600		13.9	97.2
601		1423	>10,000
602		483.5	>10,000
603		196.6	4442.0
604		>10,000	>10000
605		>10000	>10000
606		297.9	>10000

607		309.7	>10000
608		29.1	
609		193.9	
610		411.5	
611		785.6	>10000
612	 Trans (S,S)	64.8	
613	 Trans (R,R)	6726.0	
614	 (dl)	32.1	

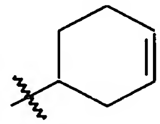
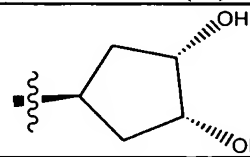
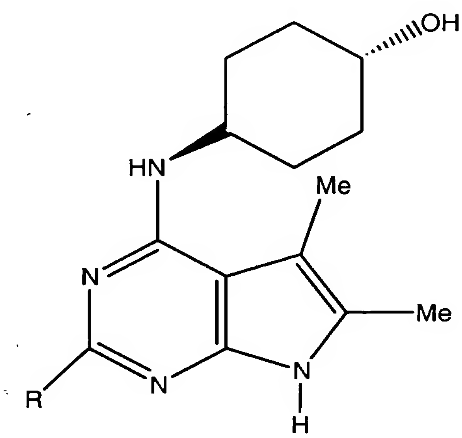
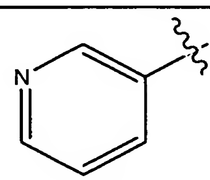
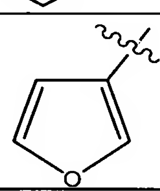
615	 (dl)	816.9	2577.0
616		34.3	

TABLE 7

Effect of C_2-Substituent



		A1	
Compound	R	Binding Ki (nM)	Yeast IC50 (nM)
700		604.5	>10000
701		157.7	763.1

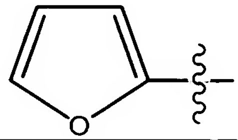
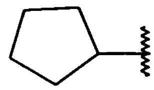
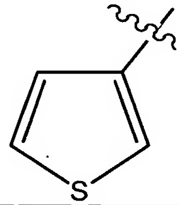
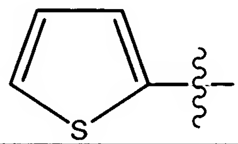
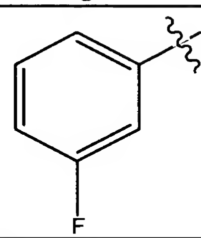
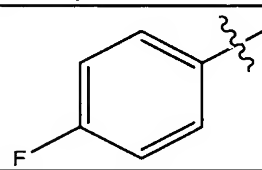
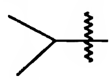
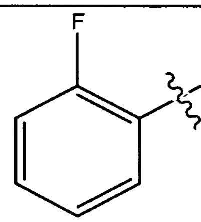
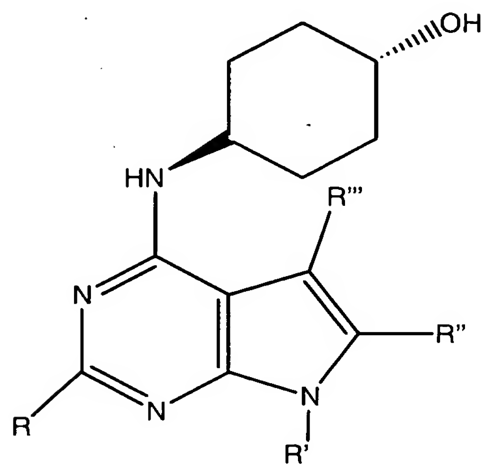
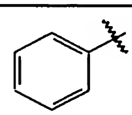
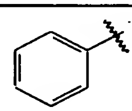
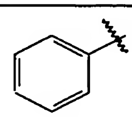
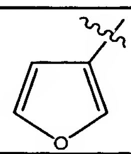
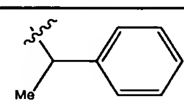
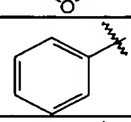
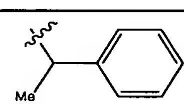
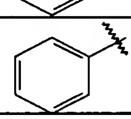
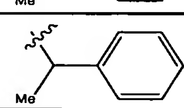
702		198.5	2782.5
703		443.6	>10000
704		61.1	297.0
705		30.1	194.7
706		19.9	
707		62.8	
708		2145	
709		48.7	

TABLE 8

Effect of Pyrrole Ring Substituent



					A1	
Compound	R	R'	R"	R'"	Binding Ki (nM)	Yeast IC50 (nM)
800		Me	Me	Me	3311	>10000
801		H	Me	H	22.3	148.3
802		H	H	Me	8.9	
803			Me	Me	2210	>10000
804			Me	Me	863.1	
805			Me	Me	4512	

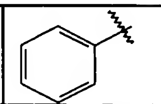
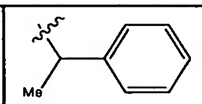
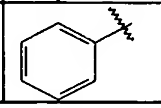
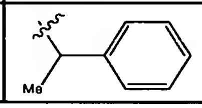
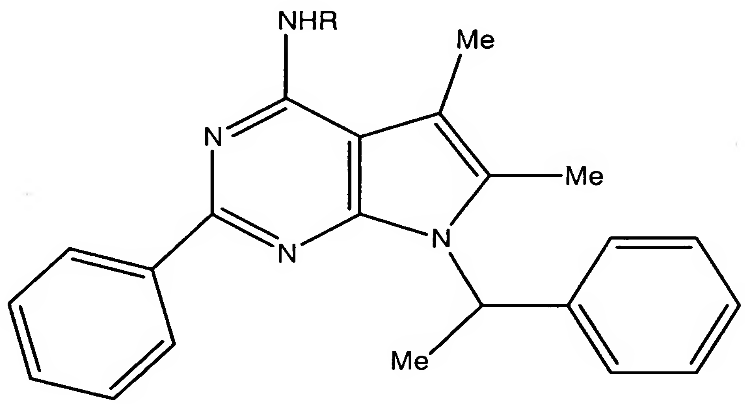
806			Me	Me	8451	
807			Me	Me	35.3	

TABLE 9



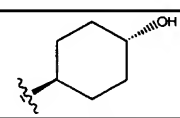
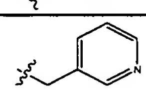
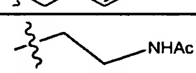
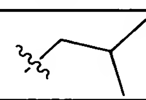
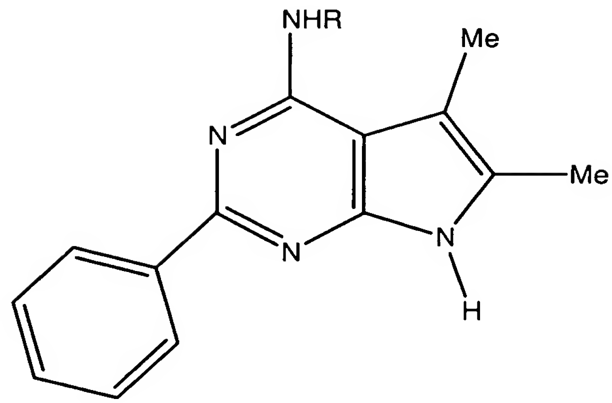
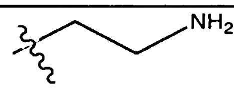
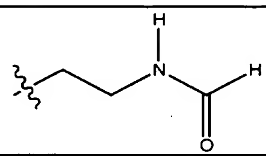
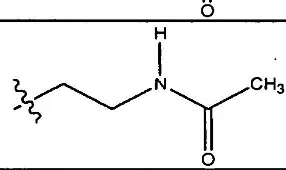
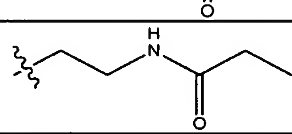
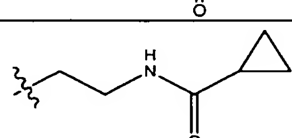
		A1	
Compound	R	Binding Ki (nM)	Yeast IC50 (nM)
900		863.1	
901		4512	
902		8451	
903		35.3	

TABLE 10

Effect of N_6-Substituent



		A1	
Compound	R	Binding Ki (nM)	Yeast IC50 (nM)
1000		17.89	>10000
1001		54.4	1865
1002		9.8	82.8
1003		26.7	195.7
1004		32.8	545.8

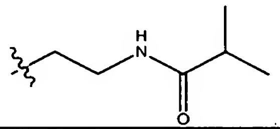
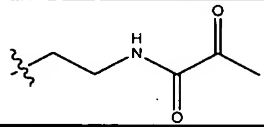
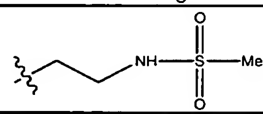
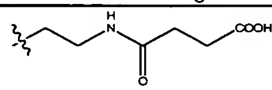
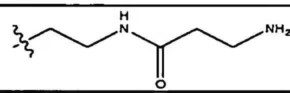
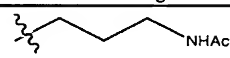
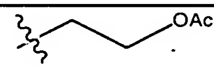
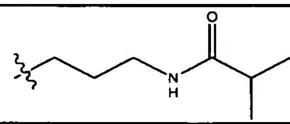
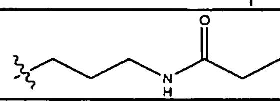
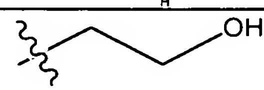
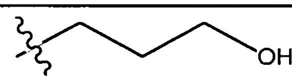
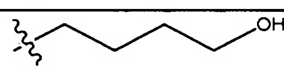
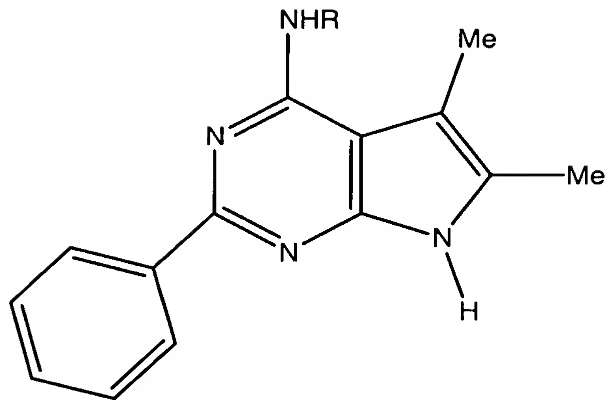
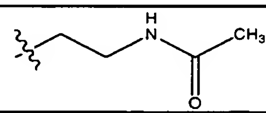
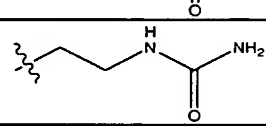
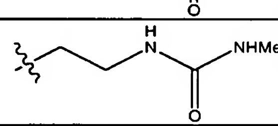
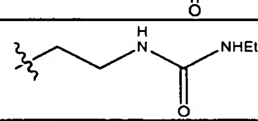
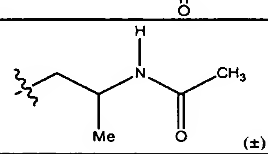
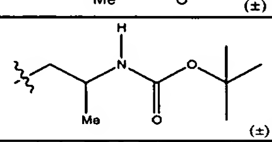
1005		147.5	3972
1006		151.7	2918
1007		692.5	>10000
1008		93.1	3217
1009		475.3	>10000
1010		674.9	9376.0
1011		121.9	2067.5
1012		233.9	3462
1013		270.1	3009.5
1014		384.9	2005
1015		179.3	3712
1016		176.1	5054

TABLE 11

Effect of N_6-Substituent



		A1	
Compound	R	Binding Ki (nM)	Yeast IC50 (nM)
1100		9.8	115.4
1101		53.9	551.0
1102		10.3	101.3
1103		71.1	3217
1104		6.5	58.7
1105		105.4	472.1

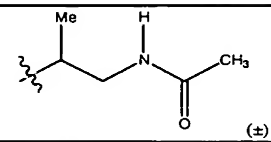
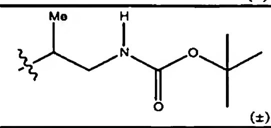
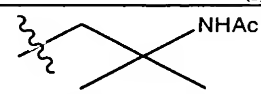
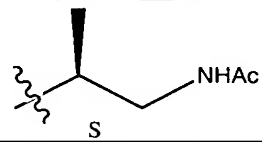
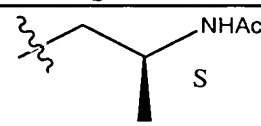
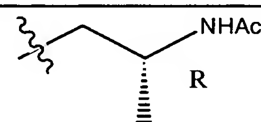
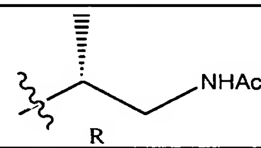
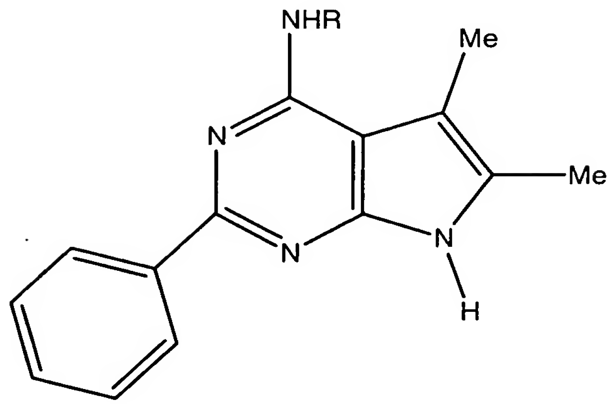
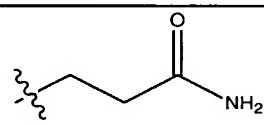
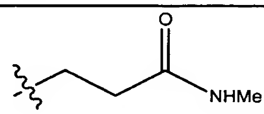
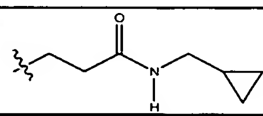
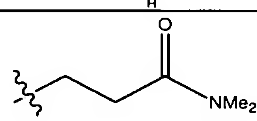
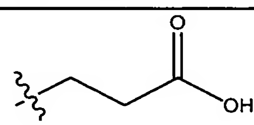
1106		27.8	162.4
1107		126.5	1297.0
1108		2.3	
1109		9.0	
1110		17.3	
1111		2.5	
1112		213	

TABLE 12

"Retro-Amide" Analogues



		A1	
Compound	R	Binding Ki (nM)	Yeast IC50 (nM)
1200	 	16.5	189.4
1201	 	7.4	45.7
1202	 	95.8	3345.0
1203	 	529.1	4040.0
1204	 	1060.0	>10000

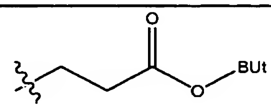
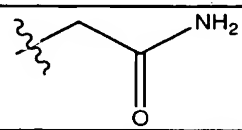
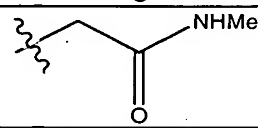
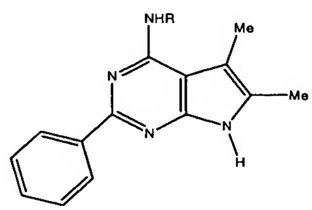
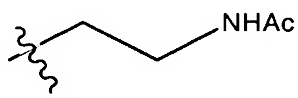
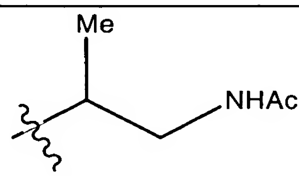
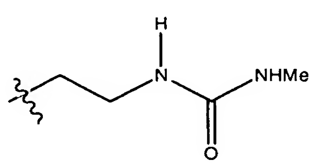
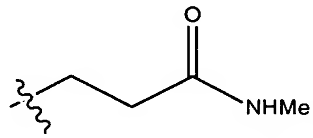
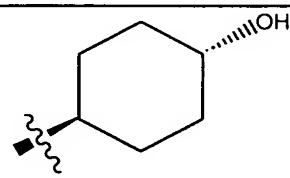
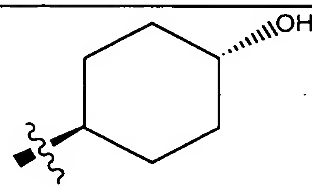
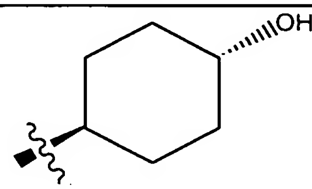
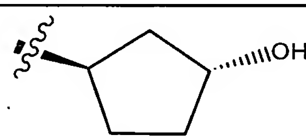
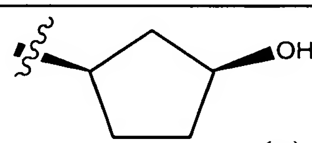
1205		1272	>10000
1206		50.8	4028
1207		48.5	701.5

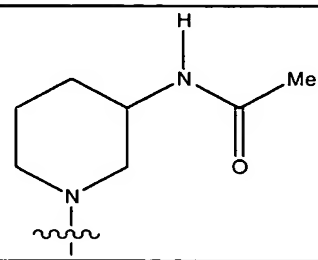
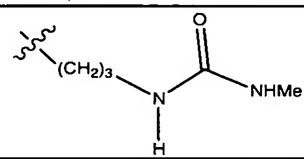
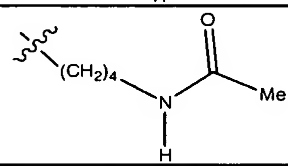
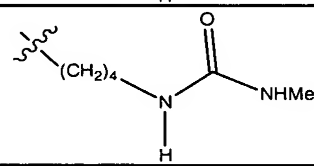
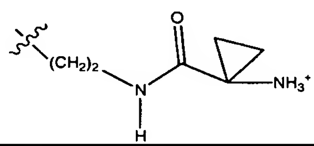
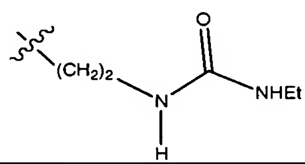
TABLE 13

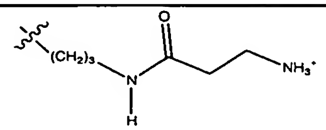
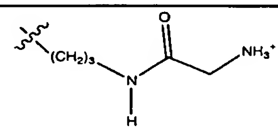
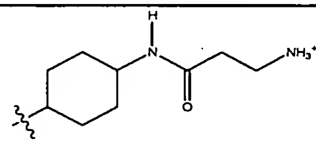
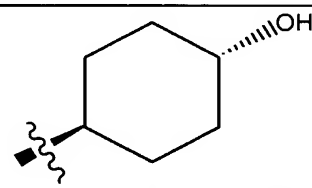
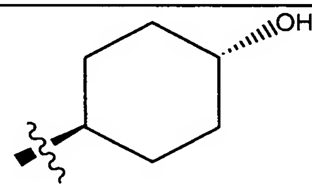
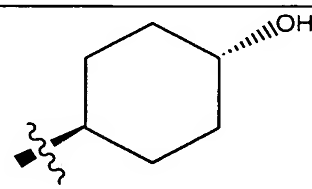
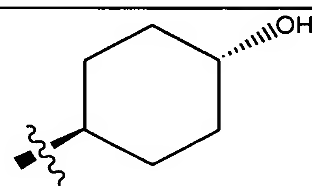
Profile of Selective Adenosine Antagonists



		Binding Ki (nM)			
Compound	R	A1	A2a	A2b	A3
1300		9.8-25.1	18.0-48.6	80.3	513.0
1301		27.8	50.7	84.6	429.8
1302		20.2	75.6	20.1	4.3

1303		17.4	111.3	120.6	44.6
1304		13.9-30.9	933.7	138.0	21.5
1305[1]		46.6	730.9	30%	9.9
1306[2]		16.4	766.3	168.3	71.7
1307	 (dl)	29.1	190.6	1143.0	3.1
1308	 (±)	180	230	670	1.0

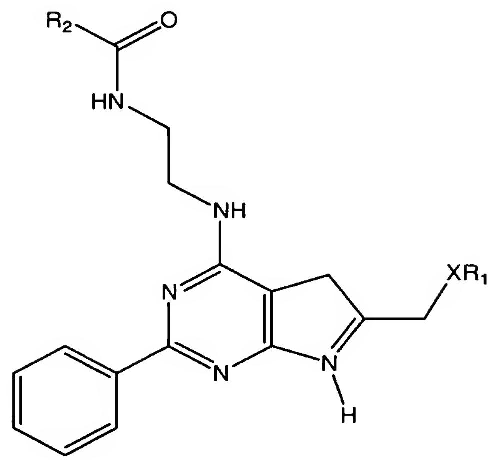
1309		40	109	109	0.3
1310		255	76%	275	≤2.6
1311		531	981	736	5.3
1312		443	2965	375	≤6.2
1313[3]		30%	65%	515	24
1314		87	204	30	0.02

1315		75,000	720,000	3,400	507
1316		333	710,000	710,000	97
1317		710,000	710,000	720,000	369
1318[4]		3.7±0.5	630± 56.4	2307± 926	630±76
1319[4.5]		1.8	206	802	270
1320[4.6]		8.0	531	530	419
1321[4.7]		8.0	131	1031	54%[8]

[1]2-thienyl-2-yl; [2]C_5-H; [3] water soluble; [4] R_5 and R_6 are hydrogen; [5] R_3 is 3-fluorophenyl; [6] R_3 is 3-chlorophenyl;[7] R_3 is 4-pyridyl; [8] % activity @ 10 μM

Table 14:

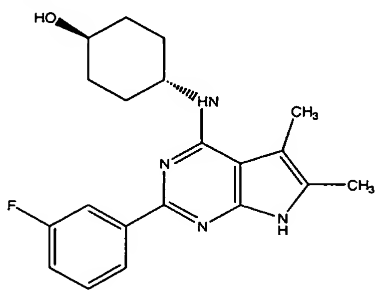
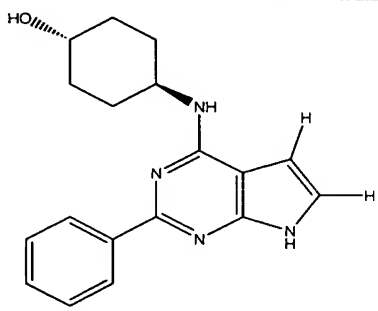
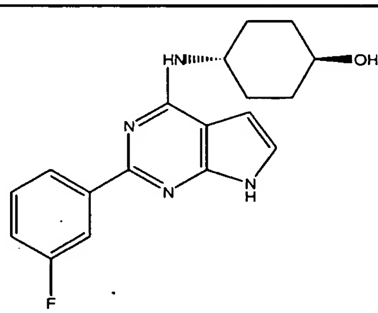
Profile of Selective A_{2b} Antagonists

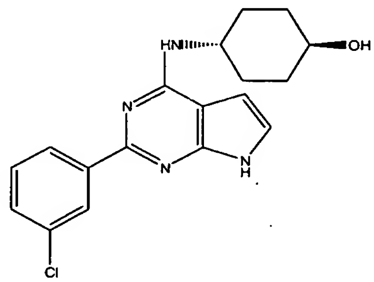
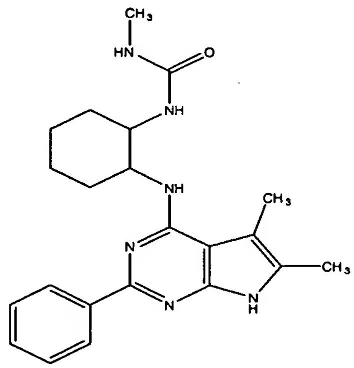
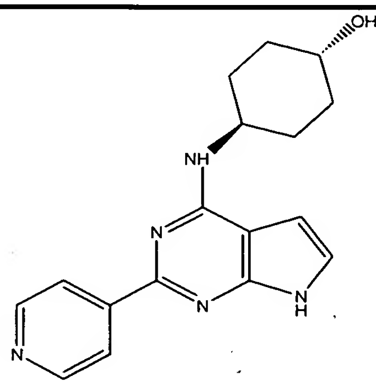


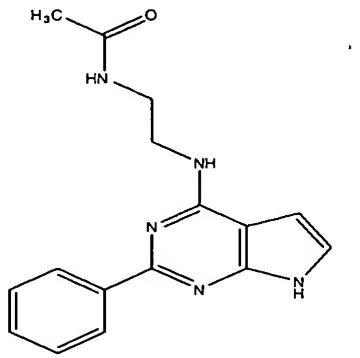
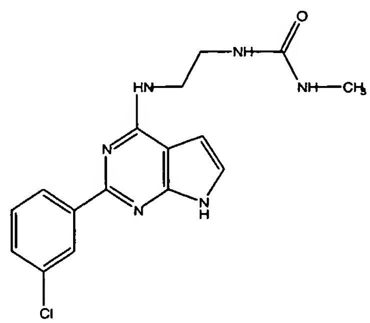
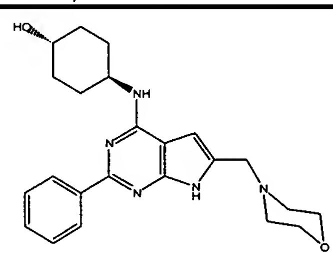
Compound	XR_1	R_2	Binding Data K_i (nM)			
			A_1	A_{2a}	A_{2B}	A_3
1400	-O-Ph	Me	41.7	21	10.3	14.6
1401	-O-Ph(p)F	Me	33	58	8.8	18
1402	-O-Ph(p)Cl	Me	825	591	22	60
1403	-N-pyridin-2-one	Me	60	41	18	48
1404	-NH-Ph	Me	49	31	4.6	57

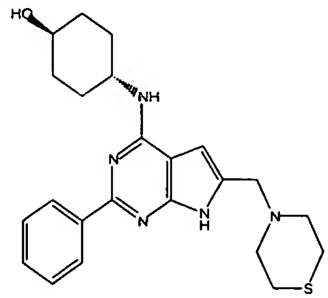
TABLE 15. Adenosine A_1 Receptor Selective Compounds

* at least 10 times more selective than other three subtypes.

Compound	Structure	Ki-A_1	Relative Ki-A_{2a}	Relative Ki-A_{2b}	Relative Ki-A_3
706		*			
1318		*			
1319		*			

1320		*			
1500		*			
1321		*			

1501		*				
1502		*				
1503		*				

1504		*			

Incorporation by Reference

All patents, published patent applications and other references disclosed herein are hereby expressly incorporated herein by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.